DETECTION AND IDENTIFICATION OF PATHOGENIC MICROORGANISMS IN NATIVE PIGEONS' FRESH FAECAL SAMPLES WITHIN UNIVERSITI TUNKU ABDUL RAHMAN (UTAR) KAMPAR CAMPUS USING SHOTGUN METAGENOMIC SEQUENCING

By

FONG GUI YING

A dissertation submitted to the Department of Biological Science,
Faculty of Science,
Universiti Tunku Abdul Rahman,
in partial fulfillment of the requirements for the degree of
Master of Science in Biological Science
August 2025

© 2026 Fong Gui Ying. All rights reserved.

This dissertation is submitted in partial fulfilment of the requirements for the degree of Master of Science in Biological Science at Universiti Tunku Abdul Rahman (UTAR). This dissertation represents the work of the author, except where due acknowledgment has been made in the text. No part of this dissertation may be reproduced, stored, or transmitted in any form or by any means, whether electronic, mechanical, photocopying, recording, or otherwise, without the prior written permission of the author or UTAR, in accordance with UTAR's Intellectual Property Policy.

ABSTRACT

DETECTION AND IDENTIFICATION OF PATHOGENIC MICROORGANISMS IN NATIVE PIGEONS' FRESH FAECAL SAMPLES WITHIN UNIVERSITI TUNKU ABDUL RAHMAN (UTAR) KAMPAR CAMPUS USING SHOTGUN METAGENOMIC SEQUENCING

Fong Gui Ying

Rock pigeon (*Columba livia*) droppings harbour diverse microorganisms, including potential pathogens. The faecal microbiota of native pigeons remains uncharacterised. This study utilised shotgun metagenomic sequencing to comprehensively analyse rock pigeon faecal microbiota and identify potential pathogens, within Universiti Tunku Abdul Rahman Kampar campus, Malaysia. The collected fresh faecal samples were subjected to total DNA and viral genome extractions, and sequenced using the Illumina NovaSeq 6000 platform. Taxonomic assignment, antimicrobial resistance (AMR) gene detection, and viral genome assembly were conducted using the CZ ID platform. Microbial diversity was predominated by bacteria, followed by eukaryotic viruses and fungi, with no archaea were detected. *Pseudomonadota* (84.44%) and *Bacillota* (15.26%) were the predominant bacterial phyla, with *Pseudomonadota* being 5.5 times more abundant, suggesting potential enteric-like issues in pigeon populations. Approximately 5.11% of the bacterial community (38 species), was identified as potential pathogens, could primarily cause human enteric and respiratory infections. Nineteen AMR genes were detected. The presence of AMR genes and potential co-circulation among pathogenic bacteria impose the risk of emergence of multidrug-resistant bacteria. Nine avian virus species were

detected. The predominant DNA virus, pigeon circovirus (73.23%) could cause immunosuppression, predisposing pigeons to secondary infections by *E. coli, K. pneumoniae*, and rotaviruses. The predominant RNA virus, rotaviruses (80.43%) could cause enteric diseases in both humans and birds. The fungal community comprised *Kazachstania* (94.11%) and *Trichosporon* (3.56%), with *K. bovina* and *T. asahii* identified as human pathogens. This study highlights the compelling need for effective pigeon control in dining areas, ventilation systems, and healthcare facilities.

Keywords: antimicrobial resistance gene; faecal microbiota; pathogens; pigoen; shotgun metagenomic; zoonotic infection

Subject Area: QR171 Microorganisms in the animal body

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor, Dr. Yap Moh Lan for her guidance and support throughout the program. With her advice and patience, I successfully overcame many obstacles I faced in this program, and had the opportunity to explore and learn a great deal of new knowledge and skills. I would also like to express my sincere appreciation to my co-supervisor, Dr. Goh Wei Lim for her valuable advice and supervision, which helped me improve myself. This work was completed with their encouragement and valuable advice.

A deep appreciation is expressed to the UTAR Research Fund for the funding provided for this research project.

My sincere appreciation also goes to my cute cats, friends, and family members for their encouragement throughout the period of study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ºC	Degree Celsius
× g	Time Gravity
%id	Percentage Identity
%Q30	Quality Score of 30
µL	Microliter
µm	Micrometer
bp	Base Pair
g	Gram
kg	Kilogram
km	Kilometer
kU/mL	Kilo Units per Milliliter
mg	Milligram
mg/mL	Milligrams per Milliliter
mL	Milliliter
V	Volt
AGE	Agarose Gel Electrophoresis
AIDS	Acquired Immunodeficiency Syndrome
AIV	Avian Influenza Virus
AMR	Antimicrobial Resistances
APMV	Avian Paramyxovirus type-1 Virus
BLAST	Basic Local Alignment Search Tool
BV-BRC	Bacterial and Viral Bioinformatics Resource Center
$CaCO_3$	Calcium Carbonate
cap	capsid
CARD	Comprehensive Antibiotic Resistance Database
DNase	Deoxyribonuclease
EDTA	Ethylenediaminetetraacetic Acid
HCl	Hydrogen Chloride
HGT	Horizontal Gene Transfer
ICTV	International Committee on Taxonomy of Viruses
ITS	Internal Transcribed Spacer

KCl	Potassium Chloride
KH_2PO_4	Potassium Dihydrogen Phosphate
L	Length of Reads
LPS	Lipopolysaccharide
MALDI-TOF	Matrix-Assisted Laser Desorption Ionization–Time of Flight
$Na_2HPO_4 \cdot H_2O$	Di-Sodium Hydrogen Phosphate Dihydrate
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NCBI	National Center for Biotechnology Information
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PE	Paired-end
PEG	Polyethylene Glycol
PiCV	Pigeon Circovirus
QC	Quality Control
RCWG	Rotavirus Classification Working Group
rep	replication-association
RGI	Resistance Gene Identifier
RNase	Ribonuclease
rPM	Reads per Million
rRNA	Ribosomal Ribonucleic Acid
TAE	Tris-acetate-EDTA
WNV	West Nile Virus
YPDS	Young Pigeon Disease Syndrome

CHAPTER 1

INTRODUCTION

Columba livia is commonly known as rock pigeon. Rock pigeon is the ancestor of the domestic pigeon that typically found in seashores (Giunchi, et al., 2012; Spennemann and Watson, 2017). With the urbanisation, rock pigeons have migrated to human-populated areas (Johnston and Janiga, 1995). In cities or towns, the beams or cavities in the buildings are the favourable places for their nesting (Bruun, n.d.). Additionally, urban areas have fewer natural predators (Pigeon Control Resource Centre, 2009). The availability of food sources is another attractive reason for their migration. Besides grains from agricultural sources, rock pigeons are often fed with bread, rice, cake or meat from human feeding, human refuse or rummaging through the garbage (Murton and Westwood, 1966; Lefebvre and Giraldeau, 1984; Jokimäki and Suhonen, 1998; Pigeon Control Resource Centre, 2009). Moreover, pigeon feeding is popular in tourist attractions such as London's Trafalgar Square, Venice's Piazza San Marco and Batu Caves (Chan, 2020; Harris, n.d.). In Malaysia, this activity has also been observed in parks and residential areas. Pigeon feeding can lead to overcrowding for food competition (Ross, 2022). The availability of food sources encourages pigeons to colonise and nest surrounding the feeding sites. Furthermore, rock pigeon has six broods annually with a higher hatching and reproduction rate in urban (Johnston and Janiga, 1995; Ovo control, 2018). Therefore, the population of rock pigeons can increase drastically in the human-inhabited areas.

The colonisation of rock pigeons leads to various issues such as noise, spreading allergens, and introducing parasites and ticks. Pigeon droppings are particularly problematic, causing damage to buildings colonised by pigeons, and posing hygiene risks to the surrounding populations, including humans (Stock and Haag-Wackernagel, 2016). The acidic property of pigeon dropping can cause unsightly soiling on the buildings.

Pigeon droppings are reservoirs for microorganisms including various pathogens (Phan, et al., 2013). Foodborne pathogenic bacteria such as *Escherichia coli*, *Salmonella*, and *Campylobacter* can cause diarrhoea and vomiting through the ingestion of contaminated food and water sources (Coburn, et al., 2007; Abulreesh, 2011; WHO, 2020). Pathogenic fungi such as *Histoplasma capsulatum* and *Cryptococcus* can cause skin infections such as histoplasmosis and cryptococcosis (Soltani, et al., 2013; CDC, 2020). Most of the detected bacteria and fungi that were isolated from pigeon droppings showed resistance to certain antibiotics, some of them even express multiple antibiotic resistances. For example, approximately 60% of diarrheagenic *E. coli* isolated from pigeon droppings in Iran were resistant to oxytetracycline (Gharajalar and Shahbazi, 2020). *Salmonella* isolated from pigeon cloaca in Bangladesh showed resistance to both ampicillin (93.1%) and tetracycline (86.2%). For fungi, 13 strains of *Cryptococcus neoformans* isolated from pigeons in Brazil were resistant to caspofungin (Costa, et al., 2010). Other than using Kirby-Bauer method that required culturing of the bacteria and fungi, molecular methods such as PCR and metagenomic sequencing were used to detect the multidrug-resistant genes.

Viruses such as pigeon circovirus, pigeon parvovirus, and rotavirus were frequently detected from pigeon droppings (Phan, et al., 2013). Avian influenza A virus (AIV) and avian paramyxovirus type-1 virus (APMV), which are pathogenic to humans are also detected in pigeons (Gronesova, Mizakova and Betakova, 2009; Kohls, et al., 2011; Phan, et al., 2013). Most of the pathogenic agents carried by pigeon droppings are able to cause infections, especially for immunocompromised individuals, premature neonates, elderly individuals, patients who recovered from major surgery, patients with AIDS, surviving organ transplantation or cancer. These individuals have weak immunity towards pathogens, especially opportunistic pathogens. The pigeon droppings might also induce allergy in some individuals (Wu, et al., 2012).

Most studies on rock pigeon faecal microbiota have targeted only specific microbial species or community, focusing either bacterial, fungal or viral taxa. For instance, *E. coli* and *Salmonella* were detected in pigeon droppings or cloaca in Western Saudi Arabia (Abulreesh, 2011; Abulreesh, 2014), Bangladesh (Bupasha, et al., 2020), Rome (Morabito, et al., 2001), and India (Wani, et al., 2004). *Campylobacter* spp. and *Chlamydia psittaci* were identified from blood and cloacal using culture-based method and PCR (Vázquez, et al., 2010). A similar study targeted only fungal species in pigeon droppings and cloacae, such as *Cryptococcus* spp. was conducted in Northeast Brazil (Costa, et al., 2010). Advanced molecular-based methods such as amplicon sequencing of 16S rRNA gene (Oh, et al., 2023) and ITS region (Wu, et al., 2012; Abulreesh, et al., 2019) have been applied to bacteria and fungi identification, respectively. However, there is no local study in Malaysia on pigeon faecal microbiota. Wild

rock pigeons inhabiting human-populated areas can act as reservoirs and vectors for potential zoonotic pathogens. Therefore, characterising the microbial diversity in local rock pigeon droppings is crucial for assessing potential public health risks, especially in the shared environments such as university campus. More importantly, integrative studies that simultaneously examine bacterial, fungal, and viral communities have not been reported. To address these gaps, this study utilised shotgun metagenomic sequencing to comprehensively identify the rock pigeon faecal microbiota, by taking UTAR Kampar campus as the study area. This approach enables the identification of both taxonomic and functional profiles, provides relative absolute abundance of each taxon, as well as allows the detection of unculturable microorganisms.

UTAR Kampar campus seems to be a favoured habitat for rock pigeons. Although pigeon feeding activity is not allowed within the campus area, rock pigeons have been observed roosting and nesting persistently in almost every building. A cafeteria located in Block C is a place where rock pigeons are observed to be nesting on the beam above the dining area. The bird droppings can always be found on dining tables, chairs, and floors. Food sold at the stalls is exposed to air. Moreover, this problem is also found in most of the buildings such as Blocks B, D, E, M, N, P, and UTAR main library.

The objectives of this study were to comprehensively detect and characterize the faecal microbiota of *Columba livia*, including bacterial, fungal, and viral communities, using shotgun metagenomic sequencing. Potential pathogens and antimicrobial resistance (AMR) genes were identified to assess

public health risks and the emergence of multidrug-resistant bacterial strains. The finding could serve as a baseline data for evaluating environmental health risks associated with pigeons around UTAR Kampar campus and the nearby UTAR Kampar hospital, which located beside to each other.

Chapter 2

Literature Review

2.1 Zoonotic Infections in Urban Birds Population

With urbanisation, several wild bird species, including pigeons (*Columba livia*), house crows (*Corvus splendens*), sparrows (*Passeridae*), and mynas (*Acridotheres tristis*), have adapted well to urban environments due to the high food availability (Alias and Hashim, 2016). These birds have also become domesticated pets. Birds serve as reservoirs for various microorganisms, including pathogenic agents (Abulreesh, 2011). This becomes problematic when pathogenic microorganisms are zoonotically transmitted from birds to humans due to the shared habitats. Transmission typically occurs through handling live or dead birds, as well as through contaminated food or water sources (Abulreesh, 2011). Occupational groups who have close contact with birds such as poultry slaughterhouse workers, bird farm workers, bird breeders, and veterinarians, are at higher risk of exposure to zoonotic pathogens (Kozdruń, Czekaj and Styś, 2015).

Avian influenza virus (AIV) is naturally found in birds and this virus, especially the highly pathogenic H5N1 strain has caused outbreaks in poultry since 1997 (Li, et al., 2004; Neumann, et al., 2010). Due to the

virus reassortment events, AIV has become fatal to humans, with a case fatality rate of 54% (upon September 2024) (Li, et al., 2004; WHO, 2024). The H5N1 strain has been isolated from crows in India and its pathogenicity was tested in mice models (Kombiah, et al., 2020). In Bangladesh, aerosolized AIV has been detected in live bird markets, coinciding with unusual cases of crow deaths (Rahman, et al., 2020). AIV was also detected in the respiratory tracts of market workers, highlighting the zoonotic transmission (Rahman, et al., 2020). Moreover, domestic pigeons and tree sparrows are also susceptible to H5N1 (Poetranto, et al., 2011; Mansour, et al., 2014). An outbreak was reported among the pigeon flocks in Egypt, with a mortality rate of 50% (Mansour, et al., 2014). The H5N1 strain isolated from tree sparrows in Indonesia was genetically similar to human strains in terms of the hemagglutinin and neuraminidase genes (Poetranto, et al., 2011).

West Nile virus (WNV) is another common virus carried by birds. It has been reported in American crows (LaDeau, et al., 2011), sparrows (O'Brien, et al., 2010), and domestic pigeons (Kim, et al., 2016). In the U.S., the abundance of American crows served as an indicator to reflect the presence of WNV due to their high susceptibility to this virus (LaDeau, et al., 2011). WNV can be transmitted by mosquitoes (Colpitts, et al., 2012). Infection with this virus can be asymptomatic or cause encephalitis and meningitis in humans (Blitvich, 2008; Colpitts, et al., 2012).

From the perspective of bacteria and fungi, bacterial infections such

as salmonellosis and colibacillosis, which are responsible for gastrointestinal disease have frequently been reported in pigeons, house crows, sparrows, and mynas (Cooper, 1996; Kamel, 2014; Fraser, et al., 2015; Blanvillain, et al., 2021; Hosseinian, 2022; Johan, et al., 2022). Psittacosis, which causes respiratory infection has also been reported in urban pigeons (Heddema, et al., 2006; Oh, et al., 2023). Fungal infections caused by *Candida* and *Rhodotorula* have been detected in both mynas and pigeons (Wu, et al., 2012; Razmyar, et al., 2014; Irga, et al., 2016; Pakshir, et al., 2019). These pathogens can be zoonotically transmitted from birds to humans through aerosolization or faecal-oral route (Hosseinian, 2022).

Healthy individuals with a strong immunity system are generally able to combat most of these pathogens. However, individuals with weak immunity such as young and elderly individuals, immunocompromised individuals, premature neonates, and patients who experience AIDS, cancer, or organ transplantation have higher risk of getting zoonotic diseases (Hosseinian, 2022). In addition to the infections caused by pathogenic bacteria and fungi carried by wild birds, the issue of antimicrobial resistance (AMR) genes among these pathogens should also be emphasised. The dissemination of AMR genes through horizontal gene transfer or mobile elements can facilitate the proliferation and circulation of resistant strains, impacting the surrounding populations that share the same ecosystems (Cao, et al., 2020).

2.2 Biological and Ecology of Rock Pigeon

Rock pigeon is scientifically known as *Columba livia*, belongs to the family *Columbidae*. This family consists of 50 genera and 352 species of pigeons and doves (Struthers, 2024). Generally, dove has smaller body size compared to pigeon (Sirhandi, et al., 2021). Rock pigeon is a common ancestor of the domestic pigeon, which originates from Western and Southern Europe, South Asia, and North Africa (Kratchenko, 2009; Kan, et al., 2010). Typically, rock pigeon is found in coastal areas and cliffs (Giunchi, et al., 2012; Spennemann and Watson, 2017). They have pale grey plumage with iridescent green or purple neck feathers, two black bars on their wings and red feet (Powlesland, 2013).

Historically, rock pigeons had significant interactions with humans. They were used to deliver messages during wartime and were involved in meat production in some countries (Chitty, 2018). Their droppings have been utilised as agricultural fertilizer (Sirhandi, et al., 2021). Pigeon racing, which involves hundreds to thousands of pigeons, is a popular activity in Europe. This activity is usually held from April to September with racing distances from 100 km to 700 km (Chitty, 2018). Pigeon fanciers often enhance pigeon diets with high-digestibility supplements and electrolytes to support long-distance flying. During pigeon racing and shows periods, diseases such as salmonellosis, campylobacteriosis, chlamydiosis as well as parasitic infections can spread among flocks (Chitty, 2018).

Due to their close interactions with humans, pigeons have been subjected to domestication. The descendants of rock pigeons within human populated areas are closely resemble rock pigeons in appearance, and some of them exhibit varying colours and patterns, such as black, brown or white of the plumage (Kan, et al., 2010; Jeyarajasingam, 2012). Domesticated rock pigeon species, which well adapted to urban environments are also known as feral pigeon (*C. livia domestica*) (Jeyarajasingam, 2012). A total of 20 pigeon species were observed in Peninsular Malaysia and Singapore (Jeyarajasingam, 2012). Pigeons can be found at low elevations and up to 1.5 km such as Cameron Highlands (Jeyarajasingam, 2012). Pigeon is territorialism (Jeyarajasingam, 2012), rarely sharing the nesting places with other bird species. The natural diet of pigeons is grain-based, including seeds, grasses, herbs, berries, fruits, and grains (Ross, 2022). Occasionally, they also consume small insects or worms (Ross, 2022). The diet of pigeons varies depending on their location. In tropical regions, pigeons obtain fruits more easily while in temperate areas, seeds are the most accessible food source (Ross, 2022). Young pigeons feed with regurgitated semi-solid food produced from the thickened lining of their parents' crops (Chitty, 2018). This substance is rich in protein and fat, providing essential nutrients for their growth and development (Ross, 2022). Domesticated rock pigeons are human commensals, which take advantages of the anthropogenic food resources and the man-made structures for roosting and nesting (Johnston and Janiga, 1995; Spennemann and Watson, 2017; Soh et al., 2021). The average body weight of an adult pigeon ranges from 315 g to 410 g, highly

depending on the availability of food in their living environment (Bruun, n.d.).

The migration of pigeons to urban areas is due to several factors. First, urban environments have fewer natural predators such as eagles (Pigeon Control Resource Centre, 2009). Second, other than trees, the cave-like structure of human buildings provides ample roosting, nesting and breeding sites (Spennemann, Pike and Watson, 2017). Rock pigeon is usually found in covered spaces, bridges, beams, eaves, and cavities. Furthermore, the urban heat protects them from freezing during cold weather (Spennemann, Pike and Watson, 2017). Most importantly, urban areas provide abundant food sources for rock pigeons (Pigeon Control Resource Centre, 2009).

Rock pigeon diet becomes more diverse with urbanisation. Besides grains obtained from agricultural sources, they are often fed with bread, rice, cake or meat from human feeding as well as refuse or rummaging through the garbage (Murton and Westwood, 1966; Lefebvre and Giraldeau, 1984; Jokimäki and Suhonen, 1998; Pigeon Control Resource Centre, 2009). Open-building restaurants are the common roosting and foraging sites. Tourist attractions such as London's Trafalgar Square and Venice's Piazza San Marco are well-known for pigeon feeding activities (Harris, n.d.). In Malaysia, pigeon feeding is a common activity in parks or residential areas. Similar behaviour is observed at Batu Caves (Chan, 2020). In Singapore, pigeons are the most abundant bird species,

commonly found near to the open food centres and refuse collection point, largely due to anthropogenic feeding (Soh, et al., 2021). These feeding activities can lead to problems such as overcrowding of pigeon flocks as they compete for food and roosting sites (Chitty, 2018; Ross, 2022).

2.3 Increasing of Rock Pigeon Population in Urban and Relative Negative Impacts

Under optimal climate conditions and adequate food sources, rock pigeon exhibits high reproductivity and hatching rates (Johnston and Janiga, 1995; Stock and Haag-Wackernagel, 2016; Ovo control, 2018). Rock pigeons are monogamous (Johnston and Janiga, 1995). On average, a pair of rock pigeons can lay two eggs at a time and produce six broods per year (Johnston and Janiga, 1995; Ovo control, 2018). Additionally, they have a lifespan of around 20 to 30 years (Chitty, 2018). The hatching rate in urban environments is higher due to the favourable conditions and the lack of natural predators (Stock and Haag-Wackernagel, 2016; Ovo control, 2018).

Rock pigeons are gregarious (Jeyarajasingam, 2012), their rapid reproductive cycle leads to a significant increase in the pigeon population (Pigeon Control Resource Centre, 2009). However, this growth brings negative impacts on humans such as noise, allergen transmission, introduction of parasites and ticks, and unsightly soiling from droppings (Gharajalar and Shahbazi, 2020).

2.4 Pigeon Droppings and the Corresponding Issues

The frequency of excretion in pigeons correlates with the volume of food consumed (Spennemann, Pike and Watson, 2017). On average, an adult pigeon can produce four to twelve kg of dropping annually (Spennemann, Pike and Watson, 2017).

Due to the body structure of pigeons, urine in their ureter is voided into the cloaca and mixed with faeces, thus, their excreta is a mixture of urine and faeces (Colvin, et al., 1966). Additionally, the excreta contain salts, phosphoric acid, nitric acid, and uric acids (Spennemann, Pike and Watson, 2017). Uric acid is also known as uricite crystals that are excreted by kidneys and naturally appears white (Spennemann, Pike and Watson, 2017; Critchley, 2021). It is an insoluble compound produced through protein metabolism (Critchley, 2021).

The acidity of the droppings can cause destructive issue on the buildings (Spennemann, Pike and Watson, 2017; Stukenholtz, et al., 2019). Many buildings are constructed from limestone, primarily composed of calcium carbonate ($CaCO_3$). This compound is highly susceptible to acid corrosion (Spennemann, Pike and Watson, 2017). The inorganic compounds in pigeon dropping provide phosphorus and nitrate sources for the growth of lichen and fungi (Armstrong, 1984; García-Rowe and Sáiz-Jiménez, 1991). This microbial community can further produce sulphuric and nitric acids, subsequently leading to building decay (Warscheid and Braams. 2000; Spennemann, Pike and Watson, 2017).

Additionally, uric acid can cause staining at the excretion site (Spennemann, Pike and Watson, 2017), therefore, increasing the economic costs such as clean-up and maintenance fees of human infrastructures (Stukenholtz, et al., 2019).

On the other hand, the acidic conditions of the pigeon dropping also encourage the growth of fungi such as *Candida*, *Rhodotorula*, and *Cryptococcus* (Costa, et al., 2010). *Cryptococcus neoformans* is a common fungus species found in pigeon droppings (Walter and Yee, 1968; Spennemann and Watson, 2017). It is a pathogenic fungus that favours acidic conditions and can cause human infections. Other than fungus, pigeon droppings serve as a reservoir of various microorganisms, potentially pathogenic microorganisms (Phan, et al., 2013). They contribute to the spread and epidemiology of pathogens, leading to human health issues (Abulreesh, et al., 2019). Individuals with compromised immune systems, including those with cancer, acquired immunodeficiency syndrome (AIDS), organ transplant recipients, premature neonates, elderly individuals, and those recovering from major surgery are particularly vulnerable to infections compared to healthy individuals (Soltani, et al., 2013).

The microbial community in pigeon droppings is significantly affected by the physical properties and pH. Physical properties such as water content and humidity of the droppings affect microbial growth. Low water content encourages the growth of fungi such as *Cryptococcus*

neoformans (Wu, et al., 2012; Vallejo, et al., 2016). The diets of pigeons and the different geographical locations also play crucial roles in the acidity of their droppings. For example, rock pigeons living near the seashores have a high-protein diet rather than a grain-based diet (McNabb, McNabb and Steeves III, 1973). Rock pigeons migrated to urban areas consume human-modified foods, such as white bread or chips. These diets increase the acidity of their dropping (McNabb, McNabb and Steeves III, 1973). Moreover, during the egg-laying phase, the pH of dropping will decrease (Spennemann, Pike and Watson, 2017). The acidity of droppings subsequently affects the microbial community, particularly the fungal community.

Furthermore, the season can also affect microbial communities. According to Dietz and collaborators (2022), the gut bacterial community of pigeons differs between summer and winter, even with a constant diet. In winter, *Lachnoclostridium* was dominant, while *Bacteroidetes* was dominant in summer. This finding reflects a seasonal correlation with pigeon metabolism (Dietz, et al., 2022).

2.5 Bacterial Community, Potential Pathogenic Bacteria and Multidrug-Resistant (MDR) Bacteria Detected in Pigeon Droppings

A diverse bacterial community has been detected in pigeon droppings through both culturing methods and molecular techniques, including 16S rRNA amplicon sequencing and shotgun metagenomic sequencing. The abundance of different bacterial phyla detected in pigeon

droppings is highly influenced by multiple factors, including diet, geographical environment, climate, age, sex, health status, and antibiotic exposure (Feye, et al., 2020).

The most frequently reported bacterial genera include *Lactobacillus*, *Escherichia*, *Salmonella*, and *Shigella* (Pasmans, et al., 2004; Abulreesh, 2011; Ji, et al., 2020; Oh, et al., 2023). Among these, *Lactobacillus* and *Escherichia* are part of the normal flora in the digestive system (Santos, et al., 2020; Khalid, et al., 2023). According to Xu and collaborators (2022), *Pseudomonadota* (especially *Escherichia*) is predominant (33% to 58%) in the small intestine of 7-day-old newly hatched squabs. Its abundance decreases with age, accompanied by an increase of *Bacillota* (especially *Lactobacillus*). This is due to the dietary shifts from pigeon milk to grains (Xu, et al., 2022). *Bacillota* are essential for digestion and energy metabolism through fermentation, therefore, it is expected that the abundance of *Bacillota* increase rapidly (over 90%) in the small intestine by day 21 of the squabs (Xi, et al., 2019; Xu, et al., 2022). A study on the microbiota of pigeon milk reported that *Bacillota* can be vertically transmitted from parents to newly hatched squabs during the lactation period via pigeon milk (Ding, et al., 2020). The dominant colonization of *Lactobacillus* also contributes to inhibition of pathogens in the pigeon intestinal tract (Xu, et al., 2022).

Healthy pigeons also harbour other bacterial phyla, such as *Actinomycetota* (ranging from 3.86% to 51%) and *Bacteroidota* (ranging

from 8.2% to 9.56%). *Actinomycetota* play a role in gut homeostasis and degradation of complex organic substances, including cellulose and chitin (Barka, et al., 2016; Binda, et al., 2018). *Bacteroidota* are involved in carbohydrates fermentation and the production of short-chain fatty acids for host energy metabolism (Zafar and Saier, 2021; Dietz, et al., 2022).

Beyond the commensal microbes, potential pathogenic bacterial have also been reported in pigeon, raising public health concerns. Pathogenic enteric bacteria *Escherichia coli*, particularly O157, has been frequently detected in pigeon droppings in various countries such as Western Saudi Arabia (Abulreesh, 2011), Rome (Morabito, et al., 2001), and India (Wani, et al., 2004). This pathogenic strain causes hemolytic uremic syndrome and intestinal infections such as hemorrhagic colitis in humans, which reported without seasonal variation in detection (Pedersen, et al., 2006; Abulreesh, 2011). *Salmonella* is another frequently reported enteric pathogen present in pigeon droppings (Pasmans, et al., 2004; Vlahović, et al., 2004; Tanaka, et al., 2005; Abulreesh, 2011). According to Pedersen and collaborators (2006), the prevalence of *Salmonella* in pigeon droppings showed no seasonal trend. However, contrasting finding reported peaks during spring and summer (Abulreesh, 2011). *Salmonella enterica* usually cause salmonellosis, a foodborne disease with symptoms such as enteric fever, diarrhoea, and bacteremia, which can be transmitted through contaminated water sources and animal products (Coburn, et al., 2007). *S. enterica* serovars Typhimurium has been isolated in pigeon dropping in Japan (Tanaka, et al., 2005), is reported with zoonotic

potential that can infect both humans and animals (Fàbrega and Vila, 2013). In the United States, approximately 40,000 enteric infections were reported annually (Fàbrega and Vila, 2013). Immunocompromised patients, young children, and elderly are at higher risk of the infection, which can be fatal (Fàbrega and Vila, 2013; Cleveland Clinic medical, 2022). *Campylobacter* is another enteric pathogen, have also been detected in pigeon dropping (Vlahović, et al., 2004). It causes campylobacteriosis, presenting with symptoms such as diarrhoea, vomiting, and fever (WHO, 2020). The infection is life-threatening for immunocompromised individuals (WHO, 2020).

Chlamydia psittaci is another pathogenic bacteria that causes a zoonotic infection, known as psittacosis. It has been reported having highly prevalent in urban pigeons. Clinical reports from various countries, including UK, US, and Japan have documented that humans were diagnosed with this disease after having contact with pigeons (Henry and Crossley, 1986; Mair-Jenkins, et al., 2018; Fukui, et al., 2021). A study in Beijing, revealed that *C. psittaci*-specific serum antibody and *C. psittaci*-specific antigen were detected in pigeons (10.0% and 26.7%, respectively) and their fanciers (24.1% and 23.9%, respectively), highlighting the zoonotic potential, particularly the individuals who have close contact with pigeons (Ling, et al., 2015). Human-to-human transmissions from infected patients have also been reported (Henry and Crossley, 1986; Mair-Jenkins, et al., 2018; Yao, et al., 2022). Individual with weakened immunity have higher risk of infection. The detection of

this pathogen in the pigeon flocks around hospital areas in Brazil has raised serious health concerns, particularly for hospitalized patients with weakened immunity (Lustosa, et al., 2024).

The emergence of multidrug-resistant (MDR) bacteria has raised a growing public health concern, with wild birds increasingly recognised as reservoirs and potential vectors for the dissemination of antimicrobial-resistant (AMR) genes across diverse ecological niches, including urban, suburban, and livestock environments (Bonnedahl and Järhult, 2014; Carroll, et al., 2015; Wu et al., 2018; Jarma, et al., 2021; Lin, et al., 2023; Esposito, et al., 2024). Although wild birds are not directly exposed to antibiotic, they can either acquire multidrug-resistant bacteria or AMR genes through contact with contaminated environments, such as wastewater, agricultural runoff, and landfills (Carroll, et al., 2015; Lin, et al., 2023; Esposito, et al., 2024). The widespread and often indiscriminate use of antibiotics in human and veterinary medicine has accelerated the development of drugs resistant strains.

Wild birds have been shown to carry and shed foodborne pathogens, particularly pathogenic *E. coli* and *Salmonella enterica*, which some strains exhibit antibiotic resistance. Consequently, their faecal microbiota have been employed to monitor AMR trends (Jarma, et al., 2021). *E. coli* is widely used as an indicator organism due to its ubiquity and ability to acquire and disseminate resistance genes (Ahmed and Gulhan, 2024; Esposito, et al., 2024). Several studies have reported the antibiotic-

resistant *E. coli* strains in pigeons. For example, 30% of Shiga toxin-producing *E. coli* isolated from pigeon droppings in Saudi Arabia were resistant to penicillin G, tetracycline, and erythromycin, while 10% were resistant to ampicillin (Abulreesh, 2011). Out of 182 diarrheagenic *E. coli* isolates from pigeon droppings in Brazil, 7.8% were resistant to ampicillin (Silva, et al., 2009). Moreover, Gharajalar and Shahbazi (2020) reported that 60% of *E. coli* isolates in Iran were resistant to oxytetracycline, an antibiotic commonly used in human medicine. The presence of resistant strains has complicated clinical treatment. Similarly, *Salmonella* isolates from pigeons have shown high resistance levels. In Saudi Arabia, 50% *Salmonella* strains were resistant to Penicillin G and erythromycin, and 62.5% were resistant to tetracycline (Abulreesh, 2011). Furthermore, Bupasha and collaborators (2020) also reported that 93.1% of *Salmonella* isolates from pigeon cloacae in live bird market in Bangladesh were resistant to ampicillin, and 86.2% were resistant to tetracycline.

By using molecular approaches, diverse AMR genes have been detected in pigeon droppings and cloacal swabs. For instance, AMR genes such as *bla* $_{\text{TEM}}$, *mec*A, and *pbp5* which resistant to β-lactams; *cat*I, which resistant to chloramphenicol; *qnr*S, which resistant to fluroquinolones; *sul*I, and *sul* II, which resistant to sulfonamides; *tet*(A), *tet*(K) , *tet*(L), *tet*(M), *tet*(O), and *tet*(Q), which resistant to tetracyclines; *aac(6')aph(2'')*, *ant(4')-Ia*, and *aph(3')-IIIa*, which resistant to aminoglycoside; and *van*A and *van*C1, which resistant to vancomycin, were detected in Costa Rica (Blanco-Peña, et al., 2017) and Czech Republic (Radimersky, et al., 2010).

Notably, AMR genes that embedded in mobile elements such as plasmids and integrons, can spread across species through horizontal gene transfer (Sánchez-Osuna, et al., 2019), potentially facilitating host switching within the microbial communities.

2.6 Fungal Community and Potential Pathogenic Fungi Detected in Pigeon Droppings

The detection of fungi in pigeon droppings involved the isolation of fungi on culturing media such as Sabouraud dextrose agar (SDA) with chloramphenicol, followed by identification based on culturing methods on selective agars (Costa, et al., 2010; Soltani, et al., 2013), molecular methods such as amplicon sequencing targeting ITS genes (Wu, et al., 2012; Abulreesh, et al., 2019; Queiroz-Aaltonen, et al., 2021), or using Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) mass spectrometry (Nualmalang, et al., 2023). The commonly reported fungi included *Cryptococcus* spp., *Candida* spp., *Rhodotorula* spp., *Saccharomyces* spp., and *Trichosporon* spp. (Costa, et al., 2010; Wu, et al., 2012; Soltani, et al., 2013; Medina, et al., 2017; Abulreesh, et al., 2019; Queiroz-Aaltonen, et al., 2021; Nualmalang, et al., 2023).

The fungal community in pigeon droppings is influenced by the pH and water content. The high urea acid provides an acidic condition that encourages the growth of fungi such as *Rhodotorula*, *Candida*, and *Cryptococcus*, which are potential pathogenic fungi (Costa, et al., 2010; Wu, et al., 2012). In healthcare settings, contaminated central venous

catheters with *Rhodotorula* can lead to bloodstream infections (Wirth and Goldani, 2012).

Candida species such as *C. albicans*, *C. gattii*, and *C. glabrate* have been detected from pigeon droppings (Wu, et al., 2012; Abulreesh, et al., 2019). *Candida* causes candidiasis, which more frequently affects women (ECOS, n.d.). These fungi can escape from the host immune system and further cause infection (Koutserimpas, et al., 2018). *C. glabrate* can cause brain abscesses and joint infection (Koutserimpas, et al., 2018; Zhu, et al., 2018).

Cryptococcus is a basidiomycete yeast commonly found in pigeon droppings (Costa, et al., 2010; Soltani, et al., 2013; Abulreesh, et al., 2019). *C. neoformans* and *C. deneofirmans* can form the *C. neoformans* species complex that causes cryptococcosis in the lung, brain or spinal cord, especially for immunocompromised individuals (Soltani, et al., 2013). AIDS patients have a higher death rate once infected with cryptococcal meningitis (Cogliati, 2013). A clinical case reported that an AIDS patient was suffering from cryptococcosis after being injured by a pigeon (Gatti, et al., 1997). In Saudi Arabia, this fungus was reported to cause abscesses and osteomyelitis in an immunocompromised patient (Al-Tawfiq and Ghandour, 2007). In 2019, the inhalation of *C. neoformans* indirectly contributed to the death of a child at Glasgow Hospital (BBC, 2019). Some *C. neoformans* and *C. gattii* strains were found to carry *CAP1* or *CAP59* genes, which encode for capsular formation (Abulreesh, et al.,

2019). The capsule protects these pathogens from the immune system and facilitates replication within the vacuole (Srikanta, Santiago-Tirado and Doering, 2014). Additionally, the phospholipase gene *PLB1*, which encodes for phospholipase production was detected in 95% (total of 19 isolates) of the isolated *Cryptococcus* (Abulreesh, et al., 2019). Phospholipase can hydrolyze the phospholipids of the cell membrane, allowing *Cryptococcus* to escape from the immune response (Noverr, et al., 2003).

The opportunistic fungi might associate with dust from dried pigeon droppings (Abulreesh, et al., 2019). Consequently, dust becomes a transmission medium for releasing pathogenic fungi into the air, potentially causing respiratory infections (Abulreesh, et al., 2019). Inhalation of dust containing fungi such as *Cryptococcus* may not immediately cause symptoms in individuals with strong immune system. However, when immunity is compromised, infection can occur (Esher, Zaragoza and Alspaugh, 2018).

2.7 Virus Community and Potential Pathogenic Viruses Detected in Pigeon Droppings

Viruses are often reported to be associated with pigeon droppings. Viruses such as pigeon circovirus and rotavirus have been detected in pigeon droppings (Phan, et al., 2013). Both are pathogenic to pigeons. Pigeon circovirus has been reported worldwide, with high prevalence rates reported among pigeon flocks in various countries such as China

(Wang, et al., 2022; Li, et al., 2024), Germany (Mankertz, et al., 2000), Italy (Coletti, et al., 2000), France (Abadie, et al., 2001), Czech Republic (Taras, et al., 2003), Belgium (Todd, et al., 2006), Poland (Stenzel and Pestka, 2014), Hungary (Phan, et al., 2013), Taiwan (Chen, et al., 2021), and Japan (Yamamoto, et al., 2015). This virus causes young pigeon disease syndrome (YPDS), primarily affecting pigeons aged from two to twelve months (Li, et al., 2024). Infected pigeons may be asymptomatic or exhibit symptoms such as weight loss, diarrhoea, and respiratory distress (Abadie, et al., 2001; Phan, et al., 2013; Loiko, et al., 2018; Wang, et al., 2022; Nath, et al., 2023; Li, et al., 2024).

Rotavirus has 11 RNA genome segments. It has a board host range and has been classified into nine groups based on the antigenic specificity of VP6 (Matthijnssens, et al., 2008; Kang, 2017; Matthijnssens, et al., 2022). Group A rotavirus infects both mammals, including humans and avian species (Kang, 2017; Matthijnssens, et al., 2022). Other groups of rotaviruses, such as groups B and C are human pathogens (Kang, 2017; Matthijnssens, et al., 2022), while groups D, F, and G are avian pathogens (Dhama, et al., 2015; Kang, 2017). Group H has been detected in humans and pigs (Yinda, et al., 2018), group I has been detected in cats and dogs (Mihalov-Kovács, et al., 2015; Phan, et al., 2017), as well as group J has been detected in bats (Bányai, et al., 2017). Rotavirus is associated with enteric diseases, with symptom such as diarrhoea (Matthijnssens, et al., 2011). Rotavirus A is the major cause of seasonal endemic diarrhoeal infections among children, especially those under five years of age

(Matthijnssens, et al., 2022). Among pigeon flocks, rotavirus A is also responsible for enteric disease and contributes to runting and stunting syndrome (Matthijnssens, et al., 2022).

In previous studies, avian influenza virus has been identified in urban pigeons (Phan, et al., 2013). Several subtypes of Avian Influenza A virus (AIV) such as H7N3, H7N6, H9N5, and H14N8 were identified from feral pigeons' oropharynx and cloaca in Košice (Gronesova, Mizakova and Betakova, 2009). Among avian influenza subtypes, H5N1 is highly pathogenic that can infect a wide range of organisms, including both birds and humans (Brown, et al., 2009). This virus has been detected from dead wild pigeons (Ellis, et al., 2004).

In addition, avian paramyxovirus type-1 virus (APMV) has been detected in pigeons worldwide (Kohls, et al., 2011; Phan, et al., 2013). The host-specific strain, pigeon-associated APMV was detected in Germany (Werner, et al., 1999; Kuiken, et al., 2017). The infection is highly depending on the immunity of the infected individual (Phan, et al., 2013). APMV can cause human infections such as conjunctivitis, fever, and chill for individuals with weak immune system (Capua and Alexander, 2004; Kuiken, et al., 2017). There have been reported for two death cases happened on the patients who suffering from severe pneumonia in USA and Netherlands (Goebel, et al., 2007; Svraka, et al., 2010; Kuiken, et al., 2017).

Moreover, antibodies specific against West Nile virus (WNV) have been detected in rock pigeons (Phan, et al., 2013). This indicates that the pigeon was infected by WNV (Phan, et al., 2013). This virus has also been identified in the brain, kidney, and spleen of feral pigeons (Monaco, et al., 2010). WNV typically causes asymptomatic infection among 80% of people (Roossinck, 2016). Individuals with weakened immune systems may experience flu-like symptoms and more severe conditions such as meningitis or paralysis (Roossinck, 2016).

2.8 Viral Consensus Genome

In this study, viral consensus genome has been assembled for viruses such as pigeon circovirus and rotavirus in which their genome sequences were detected in high completeness.

2.8.1 Pigeon Circovirus

Pigeon circovirus (PiCV) is a small, non-enveloped virus with circular single-stranded DNA genome. PiCV belongs to the family *Circoviridae*. According to International Committee on Taxonomy of Viruses (ICTV), this family consists of two genera, Cyclovirus, with both vertebrate and invertebrates as hosts, while, Circovirus, with natural hosts such as mammals including humans, birds, and fish (Breitbart, et al., 2017). There are more than 70 species classified under this family (Breitbart, et al., 2017).

From genomic perspective, they have two major open reading frames (ORFs) that encode for replication-association (*rep*) protein and capsid (*cap*) protein. *Rep* gene is more conserved than the *cap* gene (Stenzel and Koncicki, 2017; Li, et al., 2024). Deletion in *cap* gene for circovirus has been discovered by Li and collaborators (2024). Correspondingly, substitutions at the amino acid level of *cap* gene were also discovered at 28 different positions (Wang, et al., 2022). These two main genes are bidirectionally transcribed, where *rep* gene is encoded on virion-sense, while *cap* gene is encoded on complementary strand (Mankertz, et al., 2000; Breitbart, et al., 2017). The genome structure of PiCV is shown in Figure 2.1 (Stenzel and Koncicki, 2017). Different species of circovirus can encode for more than two ORFs. PiCV has additional three ORFs that encode for capsid proteins (Mankertz, et al., 2000; Breitbart, et al., 2017).

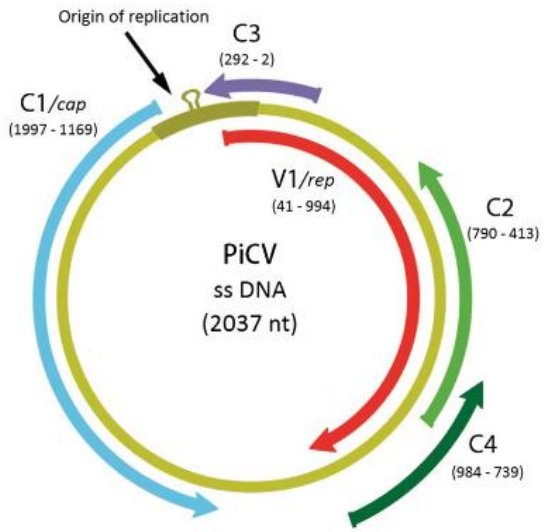


Figure 2.1: Pigeon Circovirus genome structure (Stenzel and Koncicki, 2017).

The origin of replication (*ori*) in the genome of PiCV is shown in

Figure 2.2. It is located between the start codon of the two major ORFs that encode the *rep* and *cap* genes, forming a stem-loop structure (Mankertz, et al., 2000; Li, et al., 2024). A nanonucleotide motif [(T/n)A(G/t)TATTAC] has been discovered at the apex of this hairpin structure (Mankertz, et al., 2000; Breitbart, et al., 2017; Stenzel and Koncicki, 2017). This nine-base sequence is a conserved region in which no mutations have been found due to its properties involved in the initiation of replication (Li, et al., 2024). The four forward repeat hexamers: 5'-GGAGCC-3' also has been discovered within or near to the inverted repeat (Mankertz, et al., 2000; Li, et al., 2024).

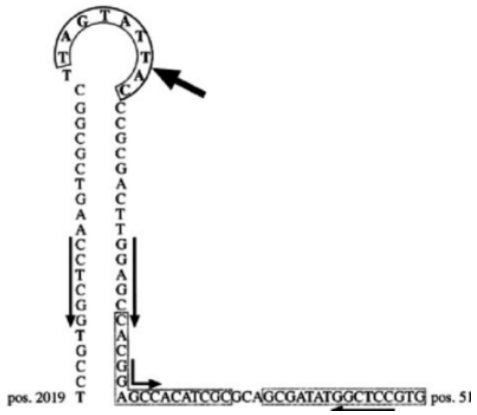


Figure 2.2: Origin of replication in the genome of pigeon circovirus (Mankertz, et al., 2000).

2.8.2 Rotavirus

Rotavirus is a non-enveloped virus with linear segmented double-stranded RNA genome (Matthijnssens, et al., 2022). It belongs to the family *Sedoreoviridae*. Rotavirus has a triple-layered icosahedral capsid, encapsidating a genome with 11 segments. Six genes encode for structural proteins, while the remaining five genes encode for non-

structural proteins. The function of these proteins is listed in Table 2.1.

Table 2.1: Eleven genome segments of rotavirus and the respective function (Matthijnssens, et al., 2008; Desselberger, 2014; Matthijnssens, et al., 2022).

Type of protein	Genome segment	Virus protein	Function
Structural protein	1	VP1	RNA-dependent RNA polymerase (RdRp)
	2	VP2	Viral inner core, necessary for RdRp activity
	3	VP3	Viral mRNA capping enzyme, guanylyltransferase, methyltransferase, etc.
	4	VP4	Spike protein for cell attachment, part of outer capsid; Protease; P type neutralization antigen
	6	VP6	Intermediate capsid that surrounded viral inner core; involved in intracellular neutralization
	9	VP7	Structural and neutralizing antigen, part of outer capsid; glycoprotein; G type neutalization antigen
Non-structural protein	5	NSP1	Antagonist of host interferon (IFN) response
	8	NSP2	NTPase & RNA-binding, formation of viroplasm
	7	NSP3	Facilitate translation of rotaviral mRNA transcripts & shut-off cellular protein synthesis
	10	NSP4	Intracellular receptor for newly viral particles
	11	NSP5	Binding partner of NSP2 in the formation of viroplasms

The inner layer of the virus is formed by a protein VP2, while the structural proteins VP1 and VP3 are enclosed within this inner layer (Matthijnssens, et al., 2008; Desselberger, 2014; Matthijnssens, et al., 2022). The intermediate capsid layer is formed by VP6, which is responsible for intracellular neutralization (Matthijnssens, et al., 2008;

Desselberger, 2014; Matthijnssens, et al., 2022). The outer layer of the virus consists of VP7, a glycoprotein and VP4, the spike protein that exhibits protease activity (Matthijnssens, et al., 2008; Desselberger, 2014; Matthijnssens, et al., 2022).

Rotavirus is classified into nine groups based on the hosts as well as the antigenic specificity of the intermediate capsid protein, VP6 (Matthijnssens, et al., 2008; Kang, 2017; Matthijnssens et al., 2022). VP6 is one of the immunogenic proteins detectable once the host is infected (Svensson, et al., 1987). Rotavirus species with more than 60% nucleotide identity in VP6 can be classified into the same species group (Johne, et al., 2011; Phan, et al., 2013). Rotaviruses belong to groups A, B, C, and H have been detected in humans, with rotavirus A serving as an enteric pathogen for both humans and avians (Kang, 2017; Matthijnssens, et al., 2022).

Rotavirus A is also classified using a binary classification system, which known as double nomenclature classification (Matthijnssens, et al., 2008). This classification primarily relies on the outer capsid proteins, VP7 and VP4, which form the antigens that induce neutralizing antibodies (Matthijnssens, et al., 2008). VP7 is referred as G serotypes due to its glycosylation function, while VP4 is referred as P serotypes for its protease-sensitivity (Kapikian, et al., 2001; Matthijnssens, et al., 2008). According to the Rotavirus Classification Working Group (RCWG), there are at least 36 G types and 51 P types (Matthijnssens, et

al., 2022). This classification is specific to rotavirus A and is based on the immunological reactivities.

Moreover, an additional classification, which involves all 11 genome segments was developed for rotavirus A, with specific nucleotide percent cutoff values. It is denoted as: Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, which represents the genotypes of segmented genes in the order: VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 (Matthijnssens, et al., 2008). This classification allows the identification of reassortment events that can occur among different strains of rotavirus A and further identify the interspecies transmission (Matthijnssens, et al., 2008). According to RCWG, to assign a sequence to genotype A, the nucleotide identity must have at least 2% above the cutoff value. The percentage identity cutoff values for each segment in the order from VP7, VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4, and NSP5 are 80%, 80%, 85%, 83%, 84%,81%, 79%, 85%, 85%, 85%, and 91%, respectively (Matthijnssens, et al., 2008).

2.9 Different Microbial Detection Methods Used in Pigeon Faecal Sample

Most existing studies on pigeon faecal microbiota have employed culture-based methods and amplicon sequencing by targeting the 16S rRNA gene for bacteria and ITS region for fungi (Costa, et al., 2010; Abulreesh, 2011; Wu, et al., 2012; Abulreesh, 2014; Oh, et al., 2023). Culture-based methods are usually applied followed by biochemical or

molecular identifications. This combining method allows the isolation of viable and culturable microorganisms for downstream analysis such as antimicrobial susceptibility test, virulence assay or PCR-based gene detection. However, this method is limited by long incubation periods, reliance on specific culture medium and incubation conditions. It has low sensitivity because many microorganisms are non-culturable or fastidious (Rudkjøbing, et al., 2016; Zhang, et al., 2021). Consequently, this method is primary used for targeted detection of known microorganism, including pathogens such as *Chlamydophila psittaci*, *Campylobacter jejuni*, *Campylobacter coli*, *Escherichia coli*, and *Cryptococcus* spp. (Costa, et al., 2010; Vázquez, et al, 2010; Abulreesh, 2014). This method is not suitable for comprehensive microbial profiling, as it may leads to underestimation of microbial diversity.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been applied to identify yeasts populations that isolated in pigeon faeces in Thailand (Nualmalang, et al., 2023). Generally, the collected faecal samples were first cultured in selective media and then proliferated in enrichment media, followed by identification using MALDI-TOF MS (Nualmalang, et al., 2023). This method offers high accuracy by differentiating closely related species (He, et al., 2022; Motteu, et al., 2022). Fungal identification is based on peptide-mass fingerprinting (PMF) or MS spectra of biomarkers, by comparing the unknown strain with the recorded PMF in the database (Singhal, et al., 2015; Nualmalang, et al., 2023). This method is limited

to culturable fungi and is strongly dependent on the quality and currency of reference databases. Its requirement for specialised training and high maintenance costs further constrains its widespread application.

Amplicon sequencing, particularly targeting the 16S rRNA and ITS regions, has been widely applied for microbiota profiling in pigeon faeces (Wu, et al., 2012; Abulreesh, et al., 2019; Ji, et al., 2020; Dietz, et al., 2022; Liu, et al., 2022). It enables rapid, large-scale analysis of microbial diversity. This method is useful for comparing microbial communities across different environments or host conditions, such as seasonal variation (Dietz, et al., 2022) or health status of host (Dietz, et al., 2022). However, it only provides relative abundances, which can misrepresent the actual microbial load in the sample. Importantly, amplification bias is unavoidable. The taxonomic resolution is commonly limited to the genus level, hindering the differentiation of genetically similar taxa, such as *Escherichia* and *Shigella* (Khot, et al., 2012; Khot and Fisher, 2013). The genomic variation that located outside the targeted regions also cannot be detected.

To overcome these limitation, shotgun metagenomic sequencing has been employed for its ability to provide a more comprehensive and accurate profile of microbial communities, including bacteria, fungi, viruses, and archaea. This method provides higher taxonomic resolution and access to functional gene information, including detection of antimicrobial resistance (AMR) genes. Furthermore, it quantifies

microbial communities, with greater accuracy by estimating relative absolute abundances of taxa. Viruses are often difficult to be cultured due to their requirement for specific host cell lines (Rudkjøbing, et al., 2016). Viral metagenomics has overcome this limitation by enabling the direct detection and characterisation of viral genomes from the samples, which has been demonstrated by Phan and collaborators (2013). Nonetheless, shotgun metagenomic has limitation in distinguish between live and dead microorganism, thus limiting its utility in viability or infectivity assessments. This limitation is still acceptable in the present study, as the primary objective is to characterise overall microbial diversity, and further identify potential pathogens to support a holistic understanding of pigeon faecal microbiota and its potential public health implications, especially for UTAR communities. Each method has distinct strengths and limitations; therefore, the integration of multiple complementary approaches is essential to achieve an in-depth and insightful research outcomes.

CHAPTER 3

MATERIALS AND METHODS

3.1 Preparation of Materials

3.1.1 10× PBS Buffer

The 10× PBS stock buffer was prepared by adding 8.0 g of sodium chloride (NaCl), 2.68 g of di-Sodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot H_2O$), 0.24 g of potassium dihydrogen phosphate (KH_2PO_4) and 0.2 g of potassium chloride (KCl) to 800 mL of distilled water. The mixture was stirred until all the powders were fully dissolved. The pH of the mixture was adjusted to 7.4 using hydrogen chloride (HCl) and sodium hydroxide (NaOH). The solution was topped up with distilled water to a final volume of 1,000 mL. To prepare 1× PBS buffer, 100 mL of the 10× stock buffer was diluted with 900 mL of distilled water.

3.1.2 50% (w/v) Polyethylene Glycol (PEG) 6000

A total of 250 g of PEG 6000 powder was added in 300 mL of distilled water. The mixture was stirred until all the powders were fully dissolved. The solution was topped up with distilled water to a final volume of 500 mL.

3.1.3 10× Tris-acetate-EDTA (TAE) Buffer

The 10× TAE stock buffer was prepared by adding 48.4 g of Tris powder, 3.7 g of ethylenediaminetetraacetic acid (EDTA) powder and 11.4 mL of glacial acetic acid in 800 mL of distilled water. The mixture was stirred until all the powders were fully dissolved. The solution was topped up with distilled water to a final volume of 1,000 mL. To prepare 1× TAE buffer, 100 mL of the 10× stock buffer was diluted with 900 mL of distilled water.

3.1.4 6× Agarose gel loading dye

A total of 1.5 mg of bromophenol blue was added into 4 mL of 50% glycerol. The mixture was stirred until all powder was fully dissolved. The solution was topped up with distilled water to a final volume of 5 mL. The mixture was then aliquoted into five microcentrifuge tubes, with 1 mL in each tube. A volume of 1 µL of ViSafe Red gel stain was added to each tube. All tubes were covered with aluminium foil to prevent light exposure and these loading dyes were stored at -20 ºC for future use.

3.2 Methodology

3.2.1 Sample Collection

The *C. livia* faecal samples were collected within the Universiti Tunku Abdul Rahman (UTAR) Kampar campus. This campus is located in Kampar, Perak, Malaysia (coordinates: 4.3085° N, 101.1537° E).

Figure 3.1 shows the map of UTAR Kampar campus. The sample collection sites included Learning Complex I (block B), Student Pavillion I (block C), Faculty of Science (block D), Faculty of Engineering and Green Technology (block E), Dewan Tun Dr. Ling Liong Sik (block M), Faculty of Information and Communication Technology (block N), Institute of Chinese Studies (block P), and the UTAR main library (block G). These building are the resting and nesting hotspots for *C. livia*. Figure 3.2 shows the set up for sampling and the collected faecal samples. Boxes covered with clean plastic sheets were placed under the resting hotspots of pigeons during nighttime. Fresh and high-water content faecal samples were processed within 12 hours upon collection and immediately subjected to genome extractions. To minimize environmental contamination, only the inner portion of each sample was used for genome extraction. White patches (uricite crystals) were excluded to ensure high purity of extracted genomes. Sterilised forceps, spatulas, and collection tubes were used throughout the process.

Two batches of sample collection were conducted from July 2022 to March 2023 and May 2023 to August 2023. Table 3.1 shows the number of collection hotspots for both batches. Each hotspot was occupied by one to six pigeons, therefore one to six droppings could be collected per hotspot, depending on the number of pigeons present. In the first batch, samples were collected twice from each hotspot, and subjected to total DNA extraction and viral genomic extraction, respectively. In the second batch, droppings were collected once from

each hotspot. Each sample was subjected to total DNA genome extraction using approximately 0.1g of faeces, and viral genome extraction from the remaining portion. No physical contact was made with the pigeons throughout the study, thus, no ethical concerns were involved.

Figure 3.1: Map of UTAR Kampar campus (UTAR, 2024). Sampling sites are circled in red colour.

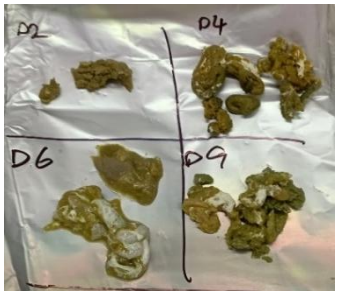
(A)  (B) 

Figure 3.2: Collection of pigeon droppings. (A) Apparatus set up placed under the hotspots for pigeon resting and nesting sites. (B) Collection of fresh pigeon droppings.

Table 3.1: Number of hotspots for pigeon dropping collections from July 2022 to March 2023 and May 2023 to August 2023.

Collection Site	Number of hotspots for pigeon dropping collections	
	July 2022 - March 2023 (1st batch)	May 2023 - August 2023 (2nd batch)
Block B	0	4
Block C	10	1
Block D	9	9
Block E	2	0
Block M	5	4
Block N	9	9
Block P	8	13
UTAR main library (Block G)	2	3
Total	45	43

3.2.2 Extraction of Total DNA Genomes

QIAGEN PowerFecal DNA kit and QIAGEN PowerFecal Pro DNA kit were used for the first and second batch extractions, respectively by following the instructions from manufacturer. Some minor modifications have been applied during the extraction to improve the concentration and purity of the extracted genome.

3.2.2.1 QIAGEN QIAamp® PowerFecal® DNA Kit (Qiagen, Germany)

The extraction was performed by following the manufacturer's instructions with slight modifications. Approximately 0.25 g of pigeon droppings was added into a bead tube. A volume of 750 µL of Solution C1 was added to the bead tube and vortexed briefly. The mixture was incubated in a water bath at 65 ºC for 10 minutes. Afterward, the tube was fixed on a vortex mixer and vortexed at maximum speed for

another 10 minutes. The sample was then centrifuged at 13,000 × g for 1 minute. The clear supernatant was transferred into a clean 2 mL collection tube and 250 µL of Solution C2 was added. The tube was vortexed briefly and incubated on ice for 5 minutes. The tube was centrifuged at 13,000 × g for 1 minute. Approximately 600 µL of clear supernatant was transferred into another clean 2 mL collection tube. A total of 200 µL of Solution C3 was added to the clear supernatant and vortexed immediately. The mixture was incubated on ice for another 5 minutes. The tube was centrifuged at 13,000 × g for 1 minute. The clear supernatant was transfer to another clean 2 mL collection tube. A total of 1.2 mL of Solution C4 was added to the supernatant and vortexed for 5 seconds.

A total of 650 µL of mixture was transferred into MB spin column. The tube was centrifuged at 13,000 × g for 1 minute and the flow-through was discarded. This step was repeated until all of the mixture passed through the column. Furthermore, 500 µL of Solution C5 was added into the column and centrifuged at 13,000 × g for 1 minute. The flow-through was discarded. An additional washing step with 500 µL of 70% (v/v) molecular grade ethanol was performed. The tube was centrifuged at 13,000 × g for 1 minute. The flow-through was discarded. The tube was centrifuged for 3 minutes at maximum speed to remove all the ethanol residue. The MB spin column was transferred into a clean 2 mL collection tube.

A volume of 50 µL distilled water preheated at 65 ºC was added to the centre of the filter membrane of the column. The column was left to stand for an additional 3 minutes at room temperature. The tube was centrifuged at 13,000 × g for 1 minute. The eluted DNA was labelled as first elution. These elution steps with distilled water were repeated for second elution. Both extracted samples were stored at -20 ºC until further use. Agarose gel electrophoresis (AGE) was performed. Concentration and purity of extracted genome was measured using Nanodrop 6000.

3.2.2.2 Qiagen QIAamp® PowerFecal® Pro DNA Kit (Qiagen, Germany)

The PowerBead Pro tube was centrifuged briefly until the beads were settled at the bottom of the tube. Approximately 0.25 g of pigeon dropping was added into the bead tube. A volume of 800 µL of Solution CD1 was added to the bead tube and vortexed briefly. The tube was fixed on a vortex mixer and vortexed at maximum speed for 10 minutes. The sample was then centrifuged at 15,000 × g for 1 minute. The clear supernatant was transferred into a clean 2 mL collection tube and 200 µL of Solution CD2 was added. The tube was vortexed briefly for 5 seconds. The tube was centrifuged at 15,000 × g for 1 minute. Approximately 600 µL of clear supernatant was transferred into another clean 2 mL microcentrifuge tube. A total of 600 µL of Solution CD3 was added to the clear supernatant and was vortexed immediately for 5 seconds.

A total of 650 µL of mixture was transferred into MB spin column. The tube was centrifuged at 15,000 × g for 1 minute and the flow-through was discarded. This step was repeated until all of the mixture passed through the column. Next, the column was placed into a clean 2 mL collection tube. A volume of 500 µL of Solution EA was added into the column and centrifuged at 15,000 × g for 1 minute. The flow-through was discarded and the column was placed back into the same 2 mL collection tube. A volume of 500 µL of Solution C5 was added into the column and centrifuged at 15,000 × g for 1 minute. The flow-through was discarded and the column was placed into a clean 2 mL collection tube. The tube was centrifuged for 3 minutes at maximum speed to remove all the ethanol residue. The MB spin column was transferred into a clean 2 mL elution tube.

A volume of 50 µL distilled water preheated at 65 ºC was added to the centre of the filter membrane of the column. The column was left to stand for an additional 3 minutes at room temperature. The tube was centrifuged at 15,000 × g for 1 minute. The eluted DNA was labelled as first elution. These elution steps with distilled water were repeated for second elution. Both extracted samples were stored at -20 ºC until further use. AGE and nanodrop analysis were performed for all extracted samples.

3.2.3 Extraction of Viral DNA and RNA Genomes

3.2.3.1 Pre-treatment of Faecal Samples and Viral Nucleic Acid Extraction

A pre-treatment process was conducted to concentrate the viral particles and remove the unprotected host's genome. This process was adapted from Phan and collaborators (2013) and Qiu and collaborators (2017), with slight modifications.

In each reaction, 4g of pooled faecal sample was mixed with 4 mL of PBS buffer in a tube containing glass beads, and heated at 74 °C for 50 minutes to inactivate the pathogens. The mixture was homogenized for 10 minutes using a vortex mixer, and centrifuged at 15,000 × g, 25 °C for 10 minutes. The supernatant was filtered through membrane (0.45 μm pore size) to remove large particles, followed by precipitation with 1/10 volume of 50% (w/v) PEG 6000 at 4 °C for 2 hours. The precipitate was centrifuged at 12,000 × g, 4 °C for 1 hour. The pellet was resuspended in 400 μL of PBS buffer. A final concentration of 0.29 kU/mL DNase and 0.01 mg/mL RNase were added to remove unprotected nucleic acids. The mixture was incubated at 37 °C for 30 minutes, followed by heat inactivation at 70 °C for 15 minutes.

The 400 μL pre-treated samples were then subjected to nucleic acid extraction using the SpinStarTM Viral Nucleic Acid Kit 1.0 (ADT Biotech, Petaling Jaya) following the manufacturer's instructions with

slight modifications.

3.2.3.2 Viral DNA and RNA Extraction using SpinStar™ Viral Nucleic Acid Kit 1.0 (ADT Biotech, Petaling Jaya)

The 400 µL pre-treated mixture that containing the viral genome was added with 100 µL of Proteinase K and 430 µL of Lysis buffer SSVL-carrier RNA in a 2 mL microcentrifuge tube. The mixture was pulse-vortexed for 15 seconds and heated at 65 °C for 10 minutes. Afterward, the mixture was centrifuged briefly. A volume of 560 µL absolute ethanol was added to the mixture. The mixture was pulse-vortexed for 15 seconds. Approximately 745 µL of the mixture was transferred into a SpinStar™ column without wetting the rim. The tube was centrifuged at 6,200 × g for 1 minutes. The flow-through was discarded and the column was placed into a clean collection tube. This step was repeated until all of the mixture passed through the column.

A volume of 500 µL of Wash Buffer 1, SSW1 was added into the column. The tube was centrifuged at 6,200 × g for 1 minutes. The flow-through was discarded and the column was placed into another clean collection tube. Subsequently, 500 µL of Wash Buffer 2, SSW2 was added into the column and centrifuged at 6,200 × g for 1 minutes. The flow-through was discarded and the column was placed back into the same collection tube. A volume of 500 µL of molecular grade absolute ethanol was added to the column, followed by centrifugation at 6,200 × g for 1 minute. The flow-through was discarded and the column was

again placed into the same collection tube. The tube was centrifuged at maximum speed for 12 minutes to remove all the ethanol residue. The SpinStar^{TM} column was transferred into a clean 1.5 mL microcentrifuge tube.

A volume of 50 µL distilled water preheated at 65 ºC was added to the center of the filter membrane of the column. The column was left to stand for an additional 5 minutes at room temperature. The tube was centrifuged at 6,200 × g for 1 minute and the eluted DNA was labelled as first elution. These elution steps were repeated for second, third and fourth elutions. All extracted samples were stored at -20 ºC until further use. AGE and nanodrop analysis were performed for all extracted samples.

3.2.3.3 Post-treatment of Extracted Viral Genome for the First Batch of Samples

Post-treatment of the extracted viral genome was purposed to obtain the pure DNA and RNA viral genome, respectively. The samples that presented clear and distinct bands on AGE, indicating the presence of non-degraded genomic materials, were used in sample pooling. Samples present with DNA and RNA viral genome were pooled into two clean 1.5 mL microcentrifuge tubes, respectively. To obtain the pure viral DNA genome, RNase with a final concentration of 0.01 mg/mL was added to one tube, while DNase with a final concentration of 0.29 kU/mL was added to another tube. Both mixtures were then

incubated at 37 °C for 30 minutes, followed by heat inactivation at 70 °C for 15 minutes. Subsequently, AGE and nanodrop analysis were performed for all treated samples.

The post-treatment procedure was only applied to the first batch samples because subsequent analyses demonstrated that it adversely affected the concentration and purity of the viral nucleic acids.

3.2.4 Analysis of Extracted Genomes using Agarose Gel Electrophoresis

3.2.4.1 Analysis of DNA Genomes in 1% (w/v) Agarose Gel

The 1% (w/v) agarose gel was prepared by adding 0.2 g of agarose powder into 20 mL of 1× TAE buffer. The mixture was heated using microwave oven until all powder was fully dissolved. The molten agarose solution was cooled to around 60 ºC in room temperature and was then poured into a gel tray with the present of eight-slotted well comb.

After solidification of agarose gel, the well comb was removed, and the gel was placed into the gel tank. The well was positioned to the negative electrode. The 1× TAE buffer was added into gel tank until fully covered the surface of agarose gel. The DNA genome was pre-stained before loaded into the gel. The gel tank was covered and connected to a power supply. The agarose gel electrophoresis was conducted at 90 Volt (V) for 40 minutes.

3.2.4.2 Analysis of RNA Genomes in 1% (w/v) Denatured Agarose Gel

This process was adapted from Aranda, LaJoie and Jorcyk (2012), with slight modifications. The 1% (w/v) denatured agarose gel was prepared by adding 0.20 g of agarose powder into 20 mL of 1× TAE buffer. The mixture was heated using microwave oven until all powder was fully dissolved. A volume of 200 µL of bleach was added to the molten agar and swirled for 5 minutes in room temperature. The mixture was heated again until all components were fully dissolved. The molten bleach-agarose solution was cooled to around 60 ºC in room temperature and was then poured into a gel tray with the present of eight-slotted well comb.

After solidification of bleach-agarose gel, the well comb was removed, and the gel was placed into the gel tank. The well was positioned to the negative electrode. The 1× TAE buffer was added into gel tank until fully covered the surface of the gel. The RNA genome was pre-stained before loaded into the gel. The gel tank was covered and connected to a power supply. The agarose gel electrophoresis was conducted at 100 V for 35 minutes.

3.2.5 Pooling and Concentration of Extracted Total DNA Samples, Viral DNA and RNA Samples

The extracted genomes were pooled and concentrated to increase the overall concentration of the extracted samples. The flowchart for the overall process of this part is shown in Figure 3.3. Samples that

presented with distinct bands on AGE were pooled and concentrated through ethanol precipitation. Initially, 1/10 volume of 3 M sodium acetate and 3 volumes of pre-cold molecular grade absolute ethanol were added to the extracted mixture. The mixture was then precipitated at -20 ºC overnight. Subsequently, the overnight mixture was centrifuged at maximum speed at 4 ºC for 30 minutes. The supernatant was removed carefully. The remaining pellet was air-dried in the laminar flow hood until all residual liquid was removed. The pellet was resuspended in 50 µL of nuclease-free water.

The total DNA samples were pooled and concentrated, named as "Pigeon_tD_1" for the first batch and "Pigeon_tD_2", for the second batch. For viral samples, the extracted samples present with viral DNA genome were pooled and concentrated, named as "Pigeon_vD_1" and "Pigeon_vD_2" for first and second batches, respectively. Samples with viral RNA genome were pooled and concentrated, named as "Pigeon_vR_1" and "Pigeon_vR_2", according to their respective batches.

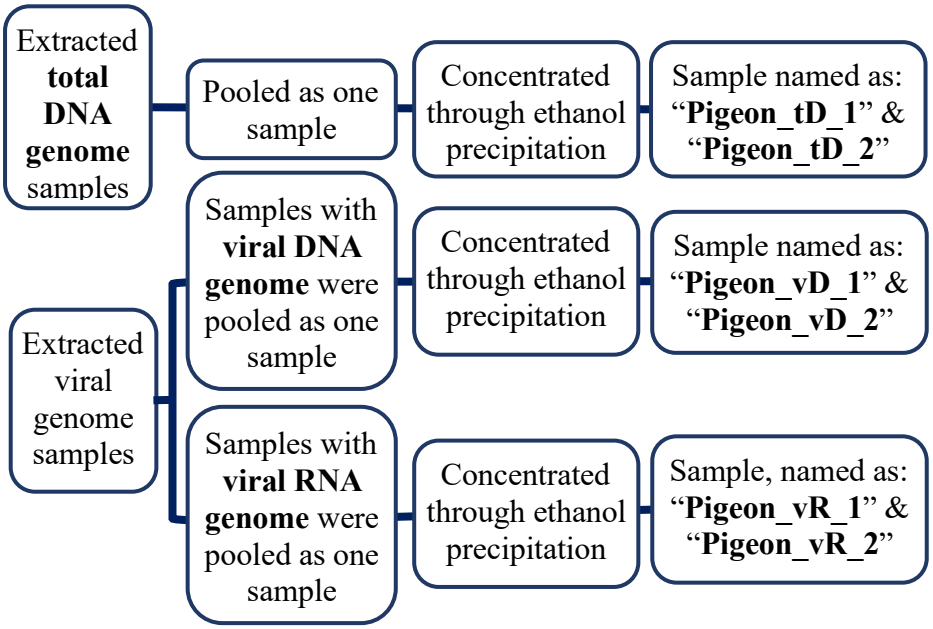


Figure 3.3: Flowchart of the process for samples pooling and concentration.

3.2.6 Quality Control (QC) Test, Library Construction and Shotgun Metagenomic Sequencing

All samples were outsourced to AGTC Genomics Sdn Bhd. A quality control (QC) test was conducted to check the quality and quantity of the samples. DNA and RNA concentrations were measured using Qubit Fluorometer 4.0, while the purity was measured using Implen NanoQuant Spectrophotometer.

Library constructions for the extracted total DNA and viral DNA samples were processed using the DNA library Prep kit (Illumina, CA, USA), following the manufacturer's instructions. For the viral RNA sample, cDNA library construction was performed using the Illumina Stranded Total RNA Prep and Ligation with Ribo-Zero Plus kit (Illumina, CA, USA) according to manufacturer's instructions. A second QC test

was conducted on the constructed libraries. The Lab Chip GX Touch™ Nucleic Acid analyzer was used to measure the average library fragment size.

All sequencings were conducted by the Illumina NovaSeq 6000 system (Illumina, CA, USA) using a paired-end 150 bp (PE150) configuration with an SP flow cell. Approximately 20 million reads per run were generated. Sample demultiplexing was performed using the Illumina DRAGEN Bio-IT platform (Illumina) v3.9. Lastly, metagenomic assembly, binning and profiling were carried out using CCMetagen, a tool that integrates multiple assembly algorithms, binning methods, and profiling. Due to the cDNA library preparation was applicable only to double-stranded RNA and positive sense RNA genomes, negative sense RNA viruses were not sequenced.

3.2.7 Concatenation of Raw Data Sets, Sequence Analysis, Diversity Analysis and Identification of Pathogens

Two data sets were generated for total DNA samples and RNA virus samples, respectively. Both data sets from similar samples were concatenated for sequence analysis. However, only a single data set was available for DNA virus sample because the first batch sample failed QC test due to the low purity of the extracted genome.

Sequence analysis was conducted using Chan Zuckerberg ID (CZ ID or IDseq, v8.3, with NCBI Index update on 6 February 2024), a

cloud-based, open-source bioinformatics platform designed for analysis of NGS data and detecting infectious disease agents (Kalantar et al. 2020). Figure 3.4 shows the workflow of sequence analysis that conducted by CZ ID. The pipelines and their respective functions are shown in Table 3.2. This platform was chosen for its no-code interface, user-friendly, continuously optimised pipelines and free accessibility, which enable researchers to conduct complex metagenomic analyses.

CZ ID utilises a series of mNGS Illumina pipelines, beginning with host filtering and QC, followed by assembly-based alignment and taxonomic identification. During data pre-processing, low-quality and low-complexity reads, ERCC reads, host read, including the *Columba livia* reference genome Cliv_2.1 (NCBI RefSeq: GCA_000337935.2) and human reads, HG38 (NCBI RefSeq: GCF_000001405.26) and T2T-CHM13 (NCBI RefSeq: GCF_009914755.1) were removed through a combination of pipelines (Kalantar, et al., 2020). Another advantage of using CZ ID is due to its integration with the National Center for Biotechnology Information (NCBI) GenBank database, allowing read alignments and taxonomic identification against curated reference sequences (Kalantar et al. 2020). Standard distilled water (dH_2O) model from CZ ID platform was used as the control for background contamination. To reduce noise and minimize the false-positive results, threshold filters were applied based on metrics such as z-score, number of reads per million (rPM), percentage identity (%id) and length of reads (L). The filtering metrics and its definition are listed in Table 3.3.

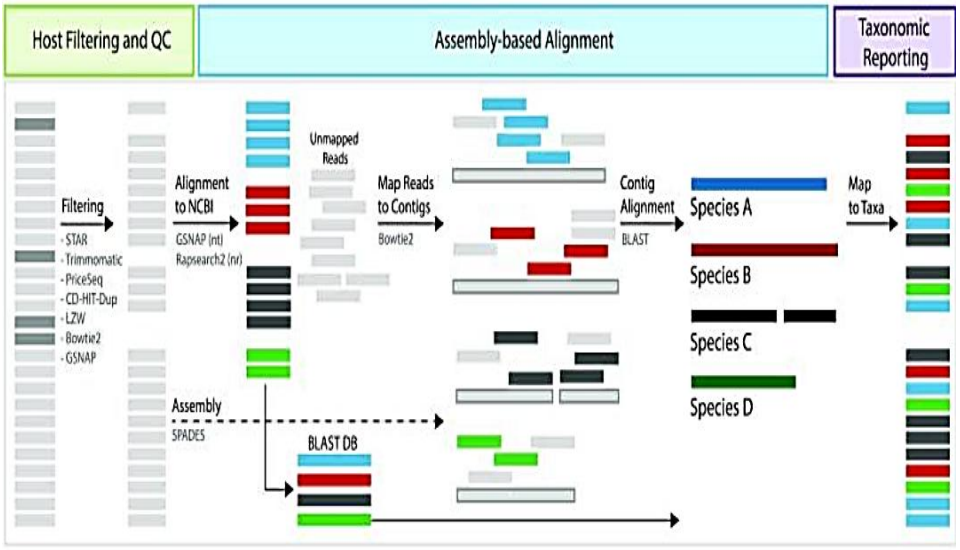


Figure 3.4: Workflow for mNGS Illumina sequence analysis of conducted by CZ ID (Kalantar, et al., 2020).

Table 3.2: The mNGS Illumina pipelines and respective functions involved in sequence analysis using CZ ID (Kalantar, et al., 2020).

Process	Pipeline	Function
Host filtering and QC (Data pre-processing)	fastp	Adapter trimming
	fastp	QC filtering: Removal of low-quality reads and low complexity reads
	Bowtie2	Removal of ERCC reads
	Kallisto	Gather host gene counts
	Bowtie2 & HISAT2	Removal of host & human reads
	CZ ID software	Removal of duplicate reads
	CZ ID software	Subsampling (subsamples to 2 million paired-end reads)
Assembly-based Alignment	Minimap2 & DIAMOND	Read alignment against NCBI NT & NR database
	SPAdes	Contig assembly
	Bowtie2	Read mapping against contigs to assess coverage
	BLASTN & BLASTX	Contigs alignment against custom nucleotide & protein databases including taxa identified with read data

Table 3.3: CZ ID Filtering Metrics and Definitions (Kalantar, et al., 2020).

Filtering Metric	Definition
Z-score	Evaluate the prevalence of microbes in the sample relative to background controls. Z-score ≥1 indicates the particular taxa is present in the sample than the negative controls
Number of reads per million (rPM)	Number of reads per million that align to reference taxon reported in NCBI database
Percentage identity (%id)	Average percentage of identity of the alignment to the NCBI database
Length of reads (L)	Average length of the alignment (including all contigs and reads) belongs to particular taxon

For bacteria and fungi communities, sequences were sorted from the concatenated total DNA data set. The highly reliable of genomic sequences were selected with the following criteria: z-score ≥ 1, rPM ≥ 10, L ≥ 100 bp, and %id ≥ 95%, as suggested by the CZ ID with minor modification (Kalantar, et al., 2020). For DNA and RNA virus communities, sequences were sorted from the respective DNA virus data set and the concatenated RNA virus data set. The genomic sequences with high reliability were selected with the following criteria: z-score ≥ 1, rPM ≥ 10, L ≥ 50 bp, and %id ≥ 85%, as suggested by the CZ ID (Kalantar, et al., 2020). The lower average length and identity match thresholds for virus community compared to bacteria and fungi community was due to the higher mutation rate and shorter genome lengths of viruses. All sequences for identified viruses were further verified by conducting BLAST search against NCBI NT and NR databases. Figure 3.5 shows the overall process for sequence analysis.

The genome sequence with low reliability for all microbial communities were excluded. The abundance of each identified taxon was referenced from the aggregated scores in the CZ ID platform. This value ranks microbial hits by evaluating species- and genus-level informations, along with relative abundance in the sample and compared to background controls. Moreover, the pathogenic microorganisms were further identified. The contig sequences for detected pathogens were repeated for BLAST search against the NCBI databases for conformation.

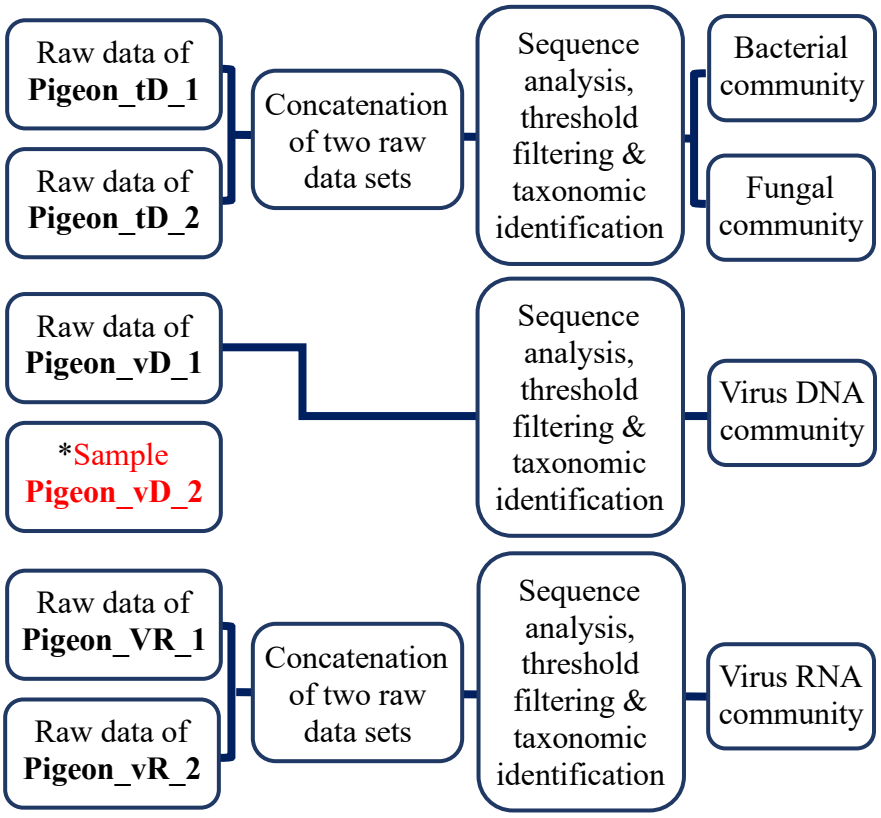


Figure 3.5: Flowchart of the process of sequence analysis. **The data set for sample Pigeon_vD_2 was not generated due to the low purity of the extracted genome.*

3.2.8 Identification of Antimicrobial Resistance (AMR) Genes

The identification of bacterial AMR genes was performed by the AMR pipeline (v1.4.2) on CZ ID. The pipelines and their respective functions are shown in Table 3.4. CZ ID utilised a series of AMR pipeline to efficiently detect bacterial AMR sequences based on the Resistance Gene Identifier (RGI) tool. The Comprehensive Antibiotic Resistance Database (CARD) (v3.2.6) was utilised by CZ ID to compare quality-controlled reads as well as contigs assembly against the reference sequences deposited in this database (Lu, et al., 2024). Figure 3.6 shows the workflow AMR gene identification conducted by CZ ID. The data pre-processing pipelines were similar to the mNGS Illumina pipelines described in Section 3.2.7.

Data were filtered based on criteria such as contig and read coverage ≥ 10%, number of reads ≥ 5 and "Perfect" cutoff score, as suggested by the CZ ID (Lu, et al., 2024). The "Perfect" score indicated identical matches between the identified sequence and the curated reference sequences and mutations deposited in CARD.

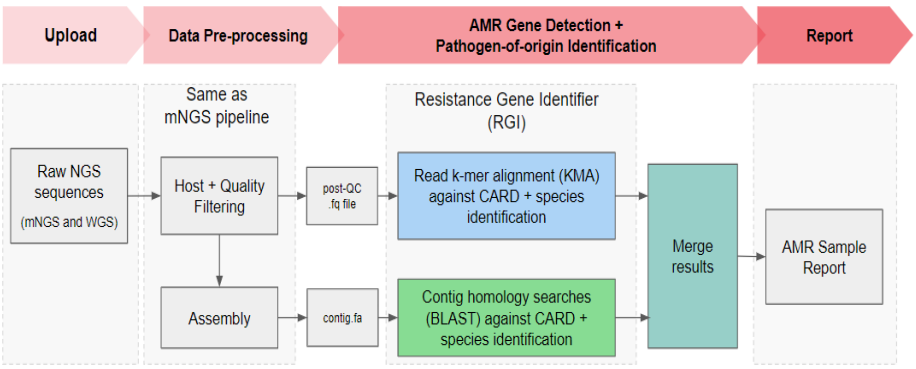


Figure 3.6: Workflow for AMR genes identification conducted by CZ ID (Lu, et al., 2024).

Table 3.4: The AMR genes identification pipelines and respective functions utilised by CZ ID (Lu, et al., 2024).

Process	Pipeline	Function
Data pre-processing	fastp, Bowtie2, Kallisto, ISAT2 & CZ ID software	Similar with mNGS Illumina pipelines shows in Table 3.2
AMR gene detection and pathogen-of-origin identification	RGI bwt	Read k-mer alignment (KMA) against CARD and identification of pathogen species based on CARD matches
	SPAdes	Contig assembly
	RGI mian	Contig homology searches (BLAST) against CARD and identification of pathogen species based on CARD matches

3.2.9 Viral Consensus Genome

The CZ ID viral consensus genome pipeline (v3.5.0) was applied to assemble the genomes of viruses, which had high genome coverage against the reference genome in the NCBI GenBank database. CZ ID utilised a series of pipeline to generate the viral consensus genome based on these reference sequences (CZ ID, n.d.). The pipelines and their respective functions for constructing the viral consensus genome in CZ ID are shown in Table 3.5.

In the present study, this function was applied to pigeon circovirus, rotavirus A and rotavirus G, the top three viruses exhibiting the highest coverage and depth. For each virus, multiple reference genome sequences showing the best BLAST hits, such as having high query cover (> 98%), low E-value (approaching zero), and high percentage of identify (>90%) were retrieved from the NCBI GenBank database.

These reference sequences were uploaded to CZ ID, used for reference-guided assembly to generate the consensus genome sequences. The resulting sequences were aligned using the ClustalW algorithm in BioEdit (version 7.2). The final consensus genome sequence was generated by merging aligned segments. The assembled sequences were subsequently deposited in the NCBI GenBank database.

Table 3.5: The viral consensus genome pipelines and respective functions utilised by CZ ID (CZ ID, n.d.).

Process	Pipeline	Function
Data pre-processing	CZ ID software	Verification of uploaded sequence file format and caps number of sequences at 100 paired-end reads
	Minimap2	Removal of human sequence
Read mapping for generation of viral consensus genome	Minimap2	Sequence alignment against the user-provided reference genome
	Trim Galore	Adapter trimming and removal of low quality and short reads (Phred score < 20 & length < 20 bp)
	Minimap2	Re-alignment of quality-filtered reads against reference genome
	iVAR	Final consensus genome call. The base pair positions with a coverage depth lesser than 10 reads are showed as Ns
	SAMtools and BCFtools	Variant calling (SNPs)

3.2.10 Genome Characterization and Phylogenetic Analysis

Open reading frames (ORFs) in the assembled genome of pigeon circovirus were identified using the NCBI ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/). For rotavirus A, genotype classification of each assembled genome segment was determined using

the ViPR Subspecies Classification Tool available in BV-BRC (https://www.bv-brc.org/app/SubspeciesClassification).

The assembled genomes were aligned with corresponding reference sequences (listed in Appendix B), which available in NCBI GenBank database for phylogenetic analysis. Multiple sequence alignments were performed using Clustal W algorithm in MEGA 11 software. The phylogenetic trees were constructed using the Neighbor-joining method, with a bootstrap test of 1,000 replications in MEGA 11 software to assess the evolutionary relationships among the virus strains.

CHAPTER 4

RESULTS

4.1. Appearance of the Collected Pigeon Dropping Samples

Two batches of sample collections were conducted. Fresh samples were processed within 12 hours upon collection and immediately subjected to genome extractions. Figure 4.1 shows different appearances of collected dropping samples. All collected faecal samples were yellow, green or dark green in colour, as shown in Figure 4.1(A). Most of the samples collected from Blocks C, D, G, N and P were watery, loose, and bright greenish-yellow or yellow in appearance, as shown in Figure 4.1(A), with red arrows indicated, and in Figure 4.1(B). One sample, which had a loose, watery consistency and was surrounded by blood, as shown in Figure 4.1(C), was also included in the genome extraction along with the other samples. The white paste, which contained uric acid was excluded for all collected samples prior to nucleic acid extraction, as shown in Figure 4.1(D).

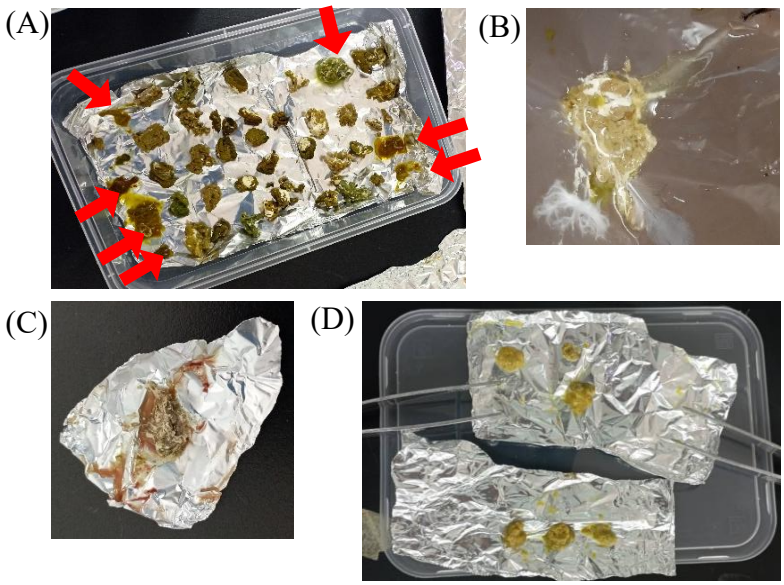
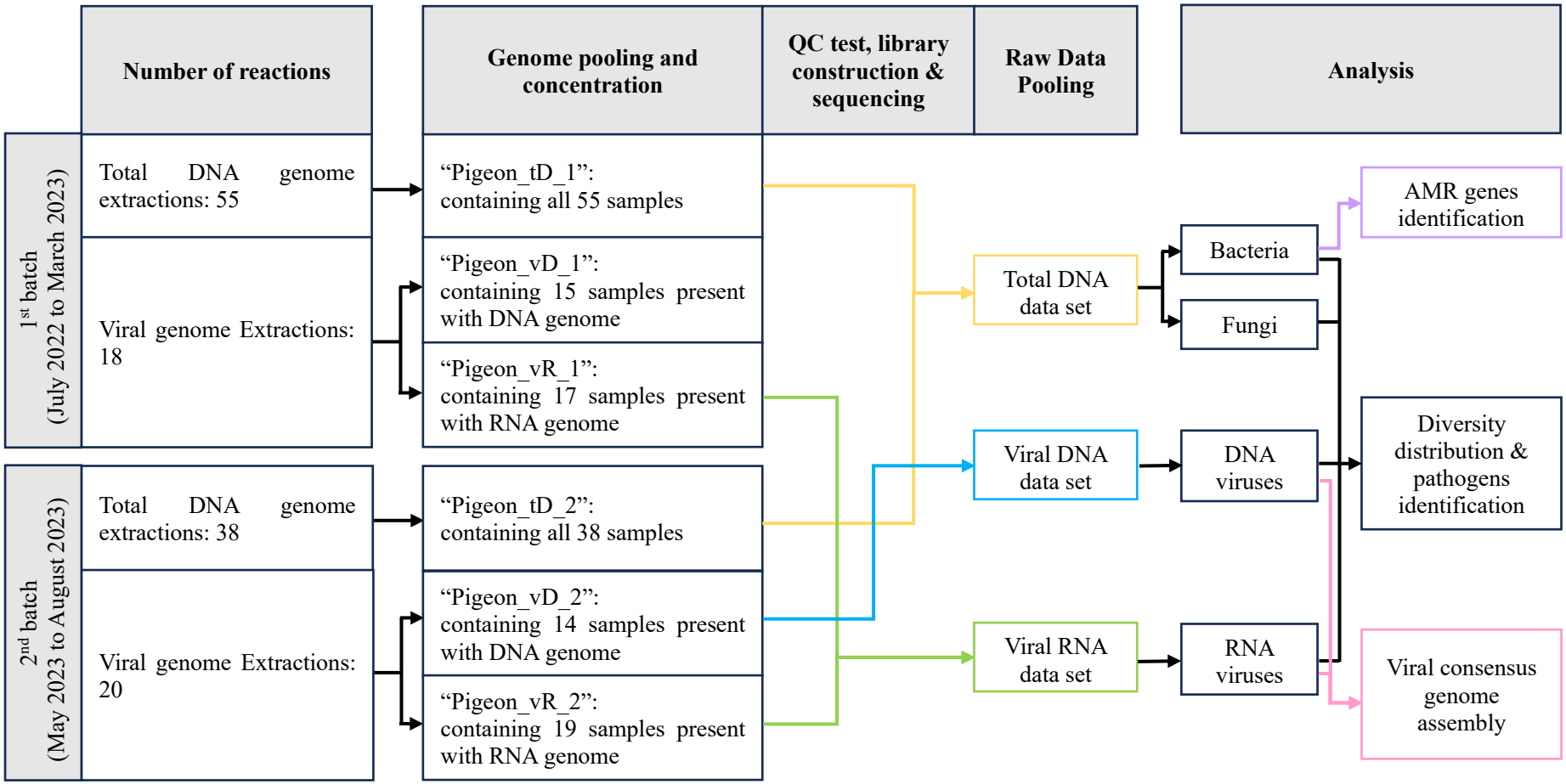


Figure 4.1: Different appearances of collected droppings samples. (A) Droppings collected within 12 hours from different blocks; the red arrows indicated watery droppings. (B) Losse, watery, yellow-greenish droppings collected in block G. (C) Losse, watery, bloody droppings collected in block P. (D) Droppings with excluded white paste.

4.2. Extraction of Nucleic Acids

The stepwise flowchart, from genome extractions to downstream data analysis, is showed in Figure 4.2. In the first batch of sample collection (July 2022 to March 2023), a total of 55 total DNA extractions and 18 viral genome extractions were performed. In the second batch of sample collection (May 2023 to August 2023), 38 total DNA extraction and 20 viral genome extractions were performed. The lower number of viral genome extractions in both batches was due to the larger amount of faecal material required per reaction, which limited the number of reactions that can be performed.

Figure 4.2: Stepwise flowchart of genome extraction, sample pooing, and raw data pooling for metagenomic analysis.



All extracted samples were initially assessed by agarose gel electrophoresis (AGE). Only the samples that presented clear and distinct bands, indicating the presence of non-degraded genomic materials, were included in subsequent genome sample pooling and concentration. The number of samples involved for each pooling is shown in Figure 4.2. This pooling step was performed to concentrate the genomic material, especially viral genome, which are typically present at low concentration due to their small genome size. For consistency, a similar pooling strategy was also applied to the total DNA samples. Figures 4.3 to 4.5 show the gel images of the pooled and concentrated total DNA samples, DNA virus samples, and RNA virus samples from two batches.

The sample, “Pigeon_tD_1” (DNA virus pool in the first batch) was found to have insufficient purity (as detailed in Section 4.3), and failed the quality control (QC) required prior to shotgun metagenomic library preparation and sequencing. Due to this failure, additional sample collections and genome extractions were conducted again, resulting in the creation of the second batch. This step was necessary to ensure adequate data quality and to generate a comprehensive microbial profile of the pigeon faecal microbiota.

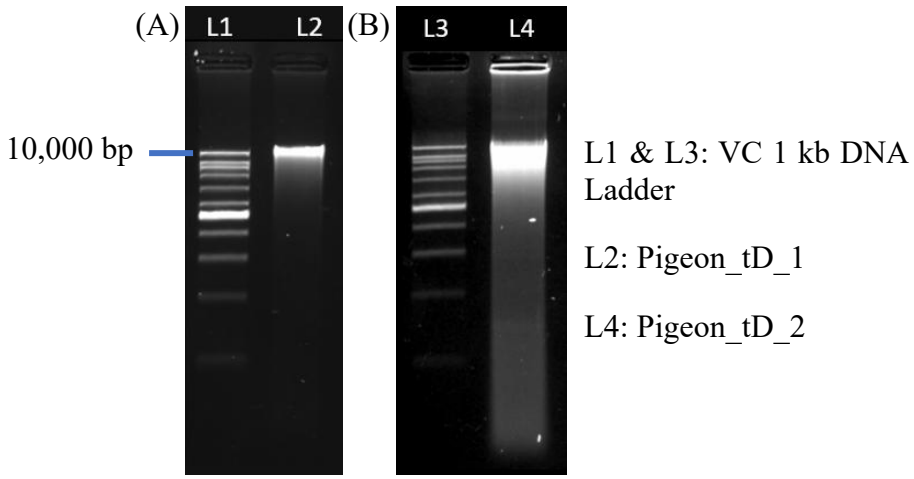


Figure 4.3: Agarose gel (1%, w/v) of pooled and concentrated total DNA samples. (A) First batch samples: Lane 1: 0.5 µL of VC 1 kb DNA Ladder; Lane 2: 1.0 µL of Pigoen_tD_1 sample. (B) Second batch samples: Lane 3: 0.5 µL of VC 1 kb DNA Ladder; Lane 4: 1.0 µL of Pigoen_tD_2 sample.

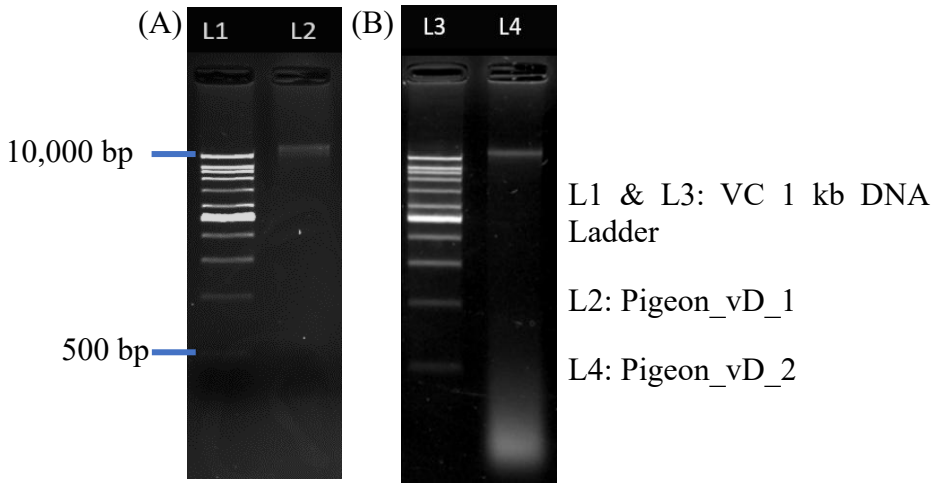


Figure 4.4: Agarose gel (1%, w/v) of pooled and concentrated DNA genomes from two batches of virus sample extractions. (A) First batch samples: Lane 1: 0.5 µL of VC 1 kb DNA Ladder; Lane 2: 1.0 µL of Pigoen_vD_1 sample. (B) Second batch samples: Lane 3: 0.5 µL of VC 1 kb DNA Ladder; Lane 4: 1.0 µL of Pigoen_vD_2 sample.

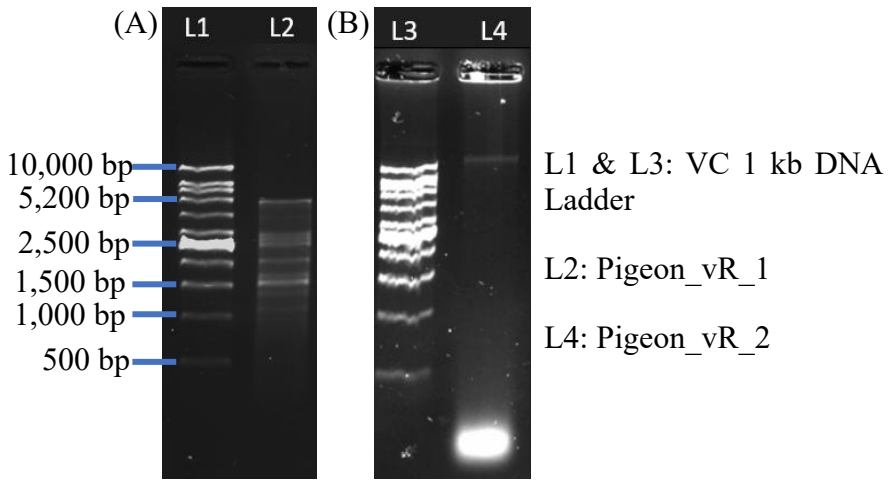


Figure 4.5: Bleach denaturing agarose gel (1%, w/v) of pooled and concentrated RNA genome from two batches of virus sample extractions. (A) First batch samples: Lane 1: 0.5 µL of VC 1 kb DNA Ladder; Lane 2: 1.0 µL of Pigoen_vR_1 sample. (B) Second batch samples: Lane 3: 0.5 µL of VC 1 kb DNA Ladder; Lane 4: 1.0 µL of Pigoen_vR_2 sample.

4.3. Library Construction and Shotgun Metagenomic Sequencing

All extracted samples were outsourced to AGTC Genomics Sdn.Bhd. for QC testing, library preparation and shotgun metagenomic sequencing. Table 4.1 and Table 4.2 present the results of sample QC and library QC, respectively.

Table 4.1: Results of sample QC.

Sample ID	Sample QC			
	Concentration (ng/µL)	A260/280	A260/230	QC
Pigeon_tD_1	43.00	1.947	1.887	Pass
Pigeon_vD_1	7.42	3.202	3.180	Fail
Pigeon_vR_1	10.40	2.687	2.581	Pass
Pigeon_tD_2	348.00	1.877	2.215	Pass
Pigeon_vD_2	6.27	2.826	2.664	Pass
Pigeon_vR_2	7.76	1.513	1.407	Pass

Table 4.2: Results of library QC.

Sample ID	Library QC		
	Concentration (ng/μL)	Average Library Size (bp)	QC
Pigeon_tD_1	12.50	631	Pass
Pigeon_vD_1	0.50	537	Fail
Pigeon_vR_1	22.60	351	Pass
Pigeon_tD_2	11.80	647	Pass
Pigeon_vD_2	1.30	555	Pass
Pigeon_vR_2	2.33	353	Pass

Sample Pigeon_vD_1 was excluded from shotgun metagenomic sequencing due to the low purity. The remaining five samples were sequenced using the Illumina Novaseq 6000 system (Illumina, CA, USA) with PE150. The statistics of raw data for forward and reverse reads of each sample are listed in Table 4.3. The percentage of bases with a quality score of 30 (%Q30) is a standard measure of sequencing data quality. It indicates that the sequencing reads have 99.9% base call accuracy. In the present study, the sequencing reads achieved 89% to 93% reads, which met the high accuracy threshold.

Table 4.3: Raw data statistics for forward and reverse reads of five samples.

Sample ID	Forward reads, R1		Reverse reads, R2	
	Total reads number	%Q30	Total reads number	%Q30
Pigeon_tD_1	26,919,495	93%	26,919,495	90%
Pigeon_vR_1	27,631,178	86%	27,631,178	91%
Pigeon_tD_2	22,154,481	93%	22,154,481	89%
Pigeon_vD_2	24,806,870	92%	24,806,870	90%
Pigeon_vR_2	26,210,217	90%	26,210,217	91%

4.4. Data Analysis

Two raw data sets for total DNA samples (Pigeon_tD_1 and Pigeon_tD_2) and RNA virus samples (Pigeon_vR_1 and Pigeon_vR_2) were concatenated, respectively. The concatenated total DNA data, concatenated RNA virus data and DNA virus data were analysed through an online metagenomic analysis platform, CZ ID. CZ ID has a series of pipelines involved QC filtering, host and human reads filtering, duplicate reads filtering followed by subsample the filled data into two million paired-end reads. Two set of filtering criteria that listed in Section 3.2.7 were applied for bacteria and fungi communities, as well as eukaryotic virus communities, respectively to reduce noise and false-positive sequences. Table 4.4 shows the number of reads remaining against the embedded filtering pipelines in CZ ID.

Table 4.4: Number of reads remaining against the embedded filtering pipelines in CZ ID.

Data set	Number of reads remaining				
	Input	QC filtering	Removal of host & human reads	Removal of duplicate reads	Subsample
Total DNA	97,791,402 (100.00%)	86,450,748 (88.40%)	85,436,540 (87.37%)	85,436,540 (87.37%)	2,189,986 (2.24%)
DNA virus	49,613,740 (100.00%)	43,791,822 (88.27%)	42,499,354 (85.66%)	42,499,354 (85.66%)	2,460,504 (4.96%)
RNA virus	10,316,814 (100.00%)	94,311,326 (87.88%)	86,533,964 (80.63%)	86,533,964 (80.63%)	7,996,460 (7.45%)

4.5. Bacterial Community

The distribution of bacterial community in pigeon droppings was analysed. The pathogenic bacteria were identified, and antimicrobial resistance genes were detected.

4.5.1. Bacterial Diversity and Pathogenic Bacteria

From the total DNA samples, a total of seven phyla of bacteria were detected. The distribution of bacterial community is shown in Figure 4.6. The bacterial community was predominantly composed of the phyla *Pseudomonadota* (84.44%) and *Bacillota* (15.26%), followed by *Actinomycetota* (0.29%) and *Bacteroidota* (~0.01%), with the remaining small proportion (<0.01%) consisting of *Campylobacterota*, *Fusobacteriota* and *Chlamydiota*.

At the genus level, a total of 112 genera, comprising 314 species of bacteria were identified, highlighting the richness and complexity of the microbiota. The predominant phylum, *Pseudomonadota*, consists of 50 genera, dominated by genera *Escherichia* (78.32% of the bacterial community), *Shigella* (1.92%), *Salmonella* (1.39%), *Klebsiella* (1.06%), *Leclercia* (0.38%), *Citrobacter* (0.32%), *Enterobacter* (0.30%), etc. *Escherichia* was the dominant genera in the bacterial community.

The following phylum was *Bacillota*. Two genera, *Lactobacillus* and *Lactobacillus* contributed as second and third dominant bacterial genera, which accounted for 8.74% and 4.41%, respectively for the

bacterial community. In the same phylum, genera including *Ligilactobacillus* (0.93%), *Enterococcus* (0.47%), *Kurthia* (0.20%), and other 30 genera were detected.

Other than the genera listed above, *Bifidobacterium* (0.03% of the bacterial community), *Sphingobacterium* (<0.01%), *Campylobacter* (<0.01%), *Fusobacterium* (<0.01%) and *Chlamydia* (<0.01%), which belong to the remaining phyla, *Actinomycetota*, *Bacteroidota*, *Campylobacterota*, *Fusobacteriota* and *Chlamydiota*, respectively, were also detected.

A number of 38 bacterial species were identified as pathogenic bacteria, contributing 5.11% of the bacterial community. The list of pathogens and their associated human infections are provided in Table 4.5. Most of these bacteria cause enteric, respiratory and urinary infections. Top five pathogenic species were *Salmonella enterica*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Shigella dysenteriae* and *Shigella boydii*. Some species, such as *Klebsiella* spp., *Acinetobacter baumannii*, *Haemophilus parainfluenzae*, *Achromobacter xylosoxidans*, *Staphylococcus aureus* and *Cronobacter sakazakii* cause co-infections. Out of 38 species, 33 of them were opportunistic pathogens that infect individuals with weakened immune systems.

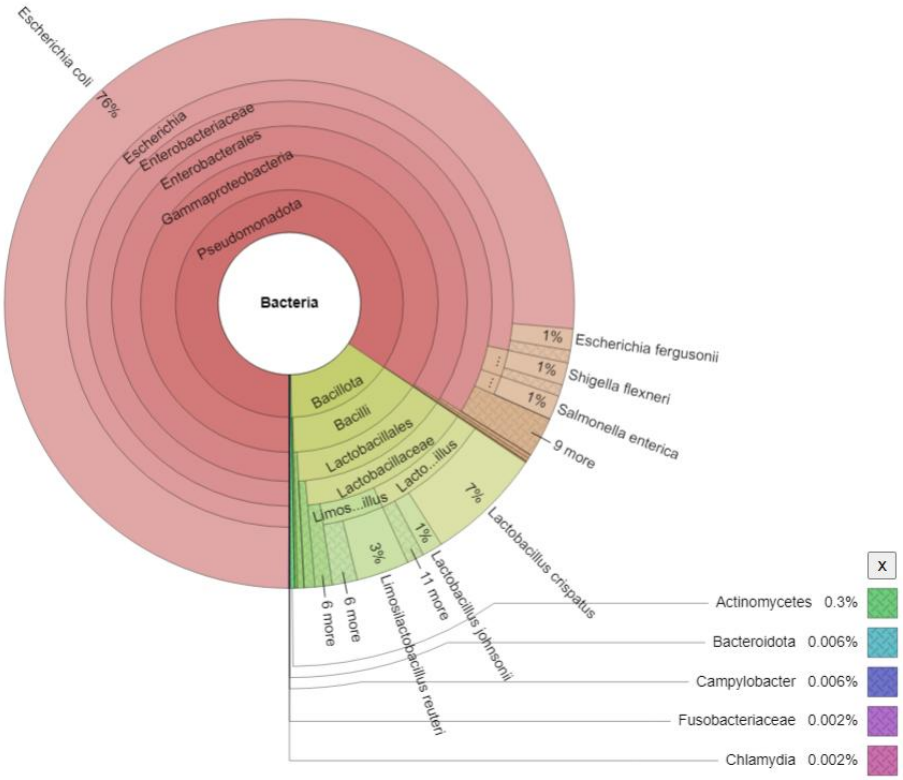


Figure 4.6: Krona chat of bacterial community detected in pigeon droppings. (Download link for the interactive krona chat: https://drive.google.com/file/d/16SDz_rH7uK-Dsc26y4U7Nw9k8Kd3TtZJ/view?usp=sharing).

Table 4.5: Pathogenic bacteria and the associated infections.

Pathogen	Infection
Aeromonas hydrophila [a]	Gastrointestinal infections such as diarrhoea
Campylobacter jejuni [a]	
Clostridioides difficile [a]	
Clostridium perfringens [a]	
Edwardsiella tarda [a]	
Listeria monocytogenes [a]	
Salmonella enterica [a]	
Shigella boydii	
Shigella dysenteriae	
Shigella flexneri	
Shigella sonnei	
Vibrio cholerae [a]	
Streptococcus suis [a]	Meningitis
Acinetobacter baumannii [a b]	Respiratory infections and meningitis
Haemophilus parainfluenzae [a]	
Chlamydia psittaci	Respiratory infections
Streptococcus pneumoniae [a]	
Stenotrophomonas maltophilia [a b]	
Klebsiella pneumoniae [a b]	Respiratory and urinary tract infections
Klebsiella aerogenes [a]	
Klebsiella oxytoca [a]	
Achromobacter xylosoxidans [a]	
Citrobacter freundii [a]	Urinary tract infections
Enterococcus faecium [a b]	
Enterococcus faecalis [a b]	
Enterococcus avium [a]	
Enterococcus gallinarum [a]	
Enterobacter cloacae [a]	
Proteus mirabilis [a]	
Providencia rettgeri [a]	
Morganella morganii [a]	
Staphylococcus saprophyticus [a]	
Staphylococcus aureus [a]	Respiratory and skin infections
Pseudomonas putida [a]	Skin infection
Staphylococcus pseudintermedius [a]	
Cardiobacterium hominis [a]	Endocarditis
Corynebacterium striatum [a]	
Cronobacter sakazakii [a]	Bloodstream and central nervous system infections

[a] *Opportunistic pathogens,* [b] *Hospital-acquired pathogens*

4.5.2. Antimicrobial Resistance (AMR) Analysis of Bacterial Community

A broad spectrum of antimicrobial resistance genes was detected, providing an overview of antibiotic resistance within the bacteria present in pigeon droppings. Table 4.6 lists the 19 AMR genes detected in this study with their respective functions and bacterial hosts. These genes are predominantly found in gram-negative bacteria and exhibit various mechanisms of antibiotic resistance.

In the perspective view of antimicrobial resistance mechanisms, 11 resistance genes can efflux antibiotics. Genes such as *AcrE*, *H-NS*, *cpxA*, *emrB*, *emrK*, *emrR*, *evgA*, *mdtE*, *mdtG*, *mdtH*, and *mdtN* encode efflux systems that facilitate the expulsion of multiple classes of antibiotics. Moreover, resistance genes such as *APH*(3')-*IIIa*, *CTX-M-1* and *DHA-1* can inactivate antibiotics like aminoglycoside, cephalosporin and cephamycin. Another resistance gene mechanism is reducing the cellular permeability to various antibiotics, which presented by *marA* gene. This gene promotes the expulsion as well as prevents influx of antibiotic. Furthermore, *QnrS1* provides protection against antibiotic such as fluoroquinolone. Lastly, *dfrA14*, *sul1* and *sul2* protect the bacteria from the effects of antibiotics including diaminopyrimidine and sulfonamide, by modifying the drug's target. Among them, *APH*(3')-*IIIa*, *QnrS1* and *sul2* are plasmid-associated genes, while *sul1* and *dfrA14* genes are integron-associated genes. These mobile elements-associated genes allow horizontal gene transfer of the AMR genes among and across the bacterial populations.

Table 4.6: Antimicrobial resistance (AMR) genes with respective functions. The bacterial host is inferred based on CARD database.

Antimicrobial Resistance Gene	Definition	Resistomes with perfect matches on CARD	Drug Class	Mechanism
acrE	Membrane fusion protein	*E. coli*, *Shigella* spp.	Cephamycin, penam, cephalaosporin, fluoroquinolone antibiotic	Antibiotic efflux
H-NS	Histone-like protein that involved in gram-negative bacteria' gene regulation	*E. coli*, *Shigella* spp.	Macrolide antibiotic, penam, cephamycin, tetracycline antibiotic, fluoroquinolone antibiotic, cephalosporin	
cpxA	Membrane-localized sensor kinase, that further promotes efflux complex expression	*E. coli*, *Shigella* spp.	Aminoglycoside antibiotic, aminocoumarin antibiotic	
emrB	Translocase	*E. coli*, *Shigella* spp.	Fluoroquinolone antibiotic	
emrK	Membrane fusion protein, involves in multidrug efflux	*E. coli*, *Shigella* spp.	Tetracycline antibiotic	
emrR	Negative regulator for EmrAB-TolC multidrug efflux pump	*E. coli*, *Shigella* spp.	Fluoroquinolone antibiotic	

Table 4.6: (cont'd)

Antimicrobial Resistance Gene	Definition	Resistomes with perfect matches on CARD	Drug Class	Mechanism
evgA	Positive regulator for efflux protein complexes	*E. coli*, *Shigella* spp.	Penam, macrolide antibiotic, tetracycline antibiotic, fluoroquinolone antibiotic	Antibiotic efflux
mdtE	Membrane fusion protein of MdtEF multidrug efflux complex	*E. coli*, *Shigella* spp.	Penam, macrolide antibiotic, fluoroquinolone antibiotic	
mdtG	Transmembrane transporter, resistance to Fosfomycin and deoxycholate	*E. coli*, *Shigella* spp.	Phosphonic acid antibiotic	
mdtH	Multidrug resistance protein	*E. coli*, *Shigella* spp.	Fluoroquinolone antibiotic	
mdtN	Multidrug resistance efflux pump, resistance to puromycin, acriflavine and tetraphenylarsonium chloride	*E. coli*, *Shigella* spp.	Nucleoside antibiotic, disinfecting agents & antiseptics	

Table 4.6: (cont'd)

Antimicrobial Resistance Gene	Definition	Resistomes with perfect matches on CARD	Drug Class	Mechanism
APH(3')-IIIa	Plasmid-encoded aminoglycoside phosphotransferase	*Campylobacter jejuni, Enterococcus* spp., *E. coli*, *Klebsiella* spp., S*taphylococcus* spp., *Streptococcus* spp.	Aminoglycoside antibiotic	Antibiotic inactivation
CTX-M-1	beta-lactamase	*E. coli*, *Klebsiella* spp., *Salmonella enterica*, *Shigella* spp.	Cephalosporin	
DHA-1	beta-lactamase	*Citrobacter* spp., *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *Morganella morganii, Salmonella enterica*, *Shigella* spp.	Cephamycin, cephalosporin	
marA	Activator protein, promote outflux and inhibit influx of antibiotic	*E. coli*, *Shigella* spp., *Klebsiella pneumoniae*	Tetracycline antibiotic, penam, monobactam, rifamycin antibiotic, glycylcycline, cephalosporin, disinfecting agents and antiseptics, penem, cephamycin, carbapenem, phenicol antibiotic, fluoroquinolone antibiotic	Antibiotic efflux; reduced permeability to antibiotic
qnrS1	Plasmid-mediated quinolone resistance protein	*Enterobacter* spp., *Escherichia* spp., *Klebsiella* spp., *Salmonella enterica*, *Shigella* spp.	Fluoroquinolone antibiotic	Antibiotic target protection

Table 4.6: (cont'd)

Antimicrobial Resistance Gene	Definition	Resistomes with perfect matches on CARD	Drug Class	Mechanism
dfrA14	Integron-encoded dihydrofolate reductase	*Aeromonas* spp., *Citrobacter* spp., *Enterobacter* spp., *Escherichia* spp., *Klebsiella* app., *Salmonella enterica*, *Shigella* spp., *Vibrio cholerae*	Diaminopyrimidine antibiotic	Antibiotic target replacement
sul1	Sulfonamide resistant dihydropteroate synthase	*Acinetobacter* spp., *Aeromonas* spp., *Citrobacter* spp., *Enterobacter* spp., *Escherichia* spp., *Klebsiella* spp., *Pseudomonas* spp., *Salmonella enterica*, *Shigella* spp., *Vibrio* spp.	Sulfonamide antibiotic	
sul2	Small plasmid associated, sulfonamide resistant dihydropteroate synthase	*Acinetobacter* spp., *Aeromonas* spp., *Citrobacter* spp., *Enterobacter* spp., *Escherichia* spp., *Klebsiella* spp., *Pseudomonas* spp., *Salmonella enterica*, *Shigella* spp., *Vibrio* spp.	Sulfonamide antibiotic	

4.6. Fungal Community

In fungal community, four fungus genera, comprising six species, were detected. Figure 4.7 shows the krona chat of the fungal community. The predominant genus was *Kazachstania*, constituting 94.11 % of the total, with two identified species, *K. unispora* and *K. bovina*. The abundance distribution for the following fungus genera were *Trichosporon* (3.56%) with species *T. asahii*, *Debaryomyces* (1.82%) with species *D. hansenii* and *Saccharomyces* (0.51%) with two identified species *S. uvarum* and *S. kudriavzevii*. *K. bovina* and *T. asahii* are potential pathogenic fungus commonly affect patients with underlying conditions.

Figure 4.7: Krona chat of fungal community detected in pigeon droppings. (Download link for the interactive krona chat: https://drive.google.com/file/d/1Qg48HTT32W6gTUp3UgSrw1kjOAI3tHhT/view?usp=sharing).

4.7. Virus Communities

Overall, the RNA virus community was more diverse than the DNA virus community. Bacteriophages were excluded from the analysis due to their complexity and the lack of a standardized classification system. Figures 4.8 and 4.9 show the abundancy distribution of the eukaryotic DNA virus and RNA virus communities, respectively.

For DNA virus community, about 73.23% was pigeon circovirus (PiCV) and 26.77% was pigeon parvovirus A. In contrast, RNA virus community has a broader diversity, comprising a total of nine genera, with eight classified species and six unclassified species. Rotavirus was the predominant genus (80.43%), including three identified species: rotavirus G, rotavirus A and rotavirus D. Unclassified *Tombusviridae*, typically associated with plant as a host, contributing 12.96% of the total abundance. The following significant genus was *Orthoreovirus* accounted for 5.85%, that infect vertebrates, including poultry, with two species, Cataraqui virus and avian orthoreovirus were identified. Additionally*, Partitiviridae* sp., targeting plants, fungi or protozoa as host, contributing 0.46%. Megrivirus B and *Megrivirus* sp. represented 0.25% of the community. The remaining minor variations were avian astrovirus, Yanbian Nodav tick virus 1, *Riboviria* sp. 2, arthropods virus belonging to *Polycipiviridae* as well as *Totiviridae* sp., which primary infect protozoa or fungi.

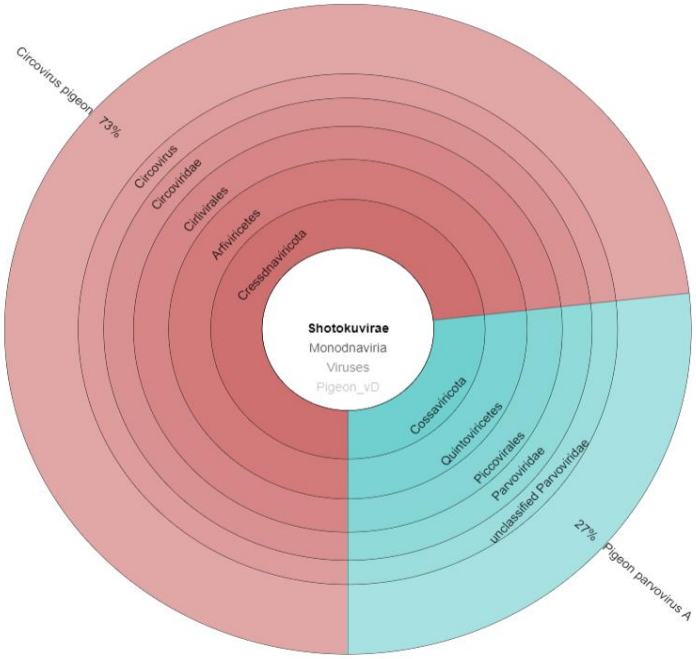


Figure 4.8: Krona chat of DNA virus community detected in pigeon droppings. (Download link for the interactive krona chat: https://drive.google.com/file/d/1lRt25jiQnAfvl_-b9Qwv6My3rEgBtpJ-/view?usp=sharing).

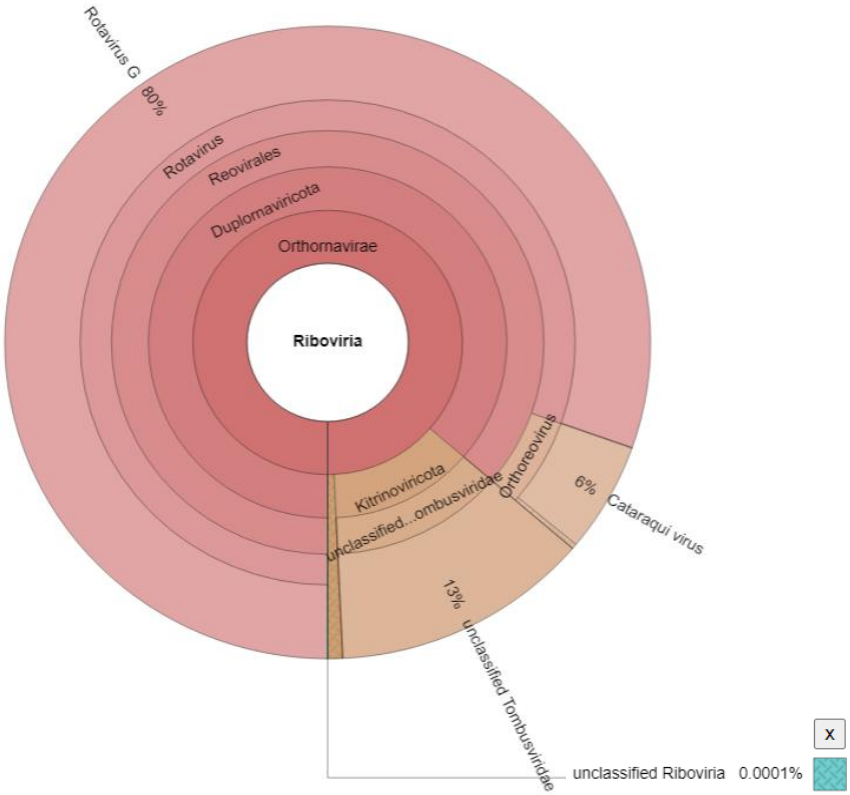


Figure 4.9: Krona chat of RNA virus community detected in pigeon droppings. (Download link for the interactive krona chat: https://drive.google.com/file/d/15ran9bELCDlQ_tB15i-mzbR5QOzD611y/view?usp=sharing).

4.8. Viral Consensus Genome

Viral consensus genome analysis was conducted on the identified viruses with high coverage in CZ ID's metagenomic NGS (mNGS) pipeline. Focusing on high-coverage viruses ensured a greater accuracy and completeness of the assembled genome, resulting in highly reliable consensus virus sequences. One DNA virus, pigeon circovirus and two RNA viruses, rotavirus A and rotavirus G were selected. Partial genomes were obtained for these three viruses.

4.8.1. Pigeon Circovirus (PiCV)

Pigeon circovirus is a DNA virus with circular single-stranded DNA genome. The reported PiCVs in the NCBI database have a full-length genome of approximately 2030 bases. In this study, consensus genome of PiCV was assembled from a total of 1,790 reads, forming a length of 2035 bases. This genome sequence was deposited in NCBI GenBank database, with the accession number, PQ570498.

The genome of PiCV consists of two main genes, which encode for replication-association (*rep*) protein and capsid (*cap*) protein, with reported lengths approximately 954 bases and 813 bases, respectively. In this study, a complete sequence of the *cap* gene, 819 bases in length, was successfully obtained. A partial complete sequence of *rep* gene, 948 bases in length, with a 21 bases gap of unidentified nucleotide, "N" was obtained.

The conserved nonanucleotide motif, 5'-TAGTATTAC-3', was found between the two ORFs that encode the *rep* and *cap* genes. Additionally, the four forward repeat hexamers, 5'-GGAGCC-3', were also detected.

Phylogenetic analysis was performed using the full-length sequence (2035 bp; listed in Appendix C) obtained in this study along with 51 full-length PiCV reference sequences deposited in NCBI to study the genetic relationship among different strains. The phylogenetic tree of full-length PiCV sequences is shown in Figure 4.10. The PiCV strain obtained in this study (indicated by a dark red circle in Figure 4.10) shows the closest genetic relationship with pigeon strain, BJ01, origin from China, with a nucleotide identity of 92.58%. The present PiCV strain also forms a monophyletic clade with various strains originating from different countries including China, Poland, and Belgium, suggesting a broad geographical distribution of genetically related PiCV strains among pigeon flocks across Asia and Europe.

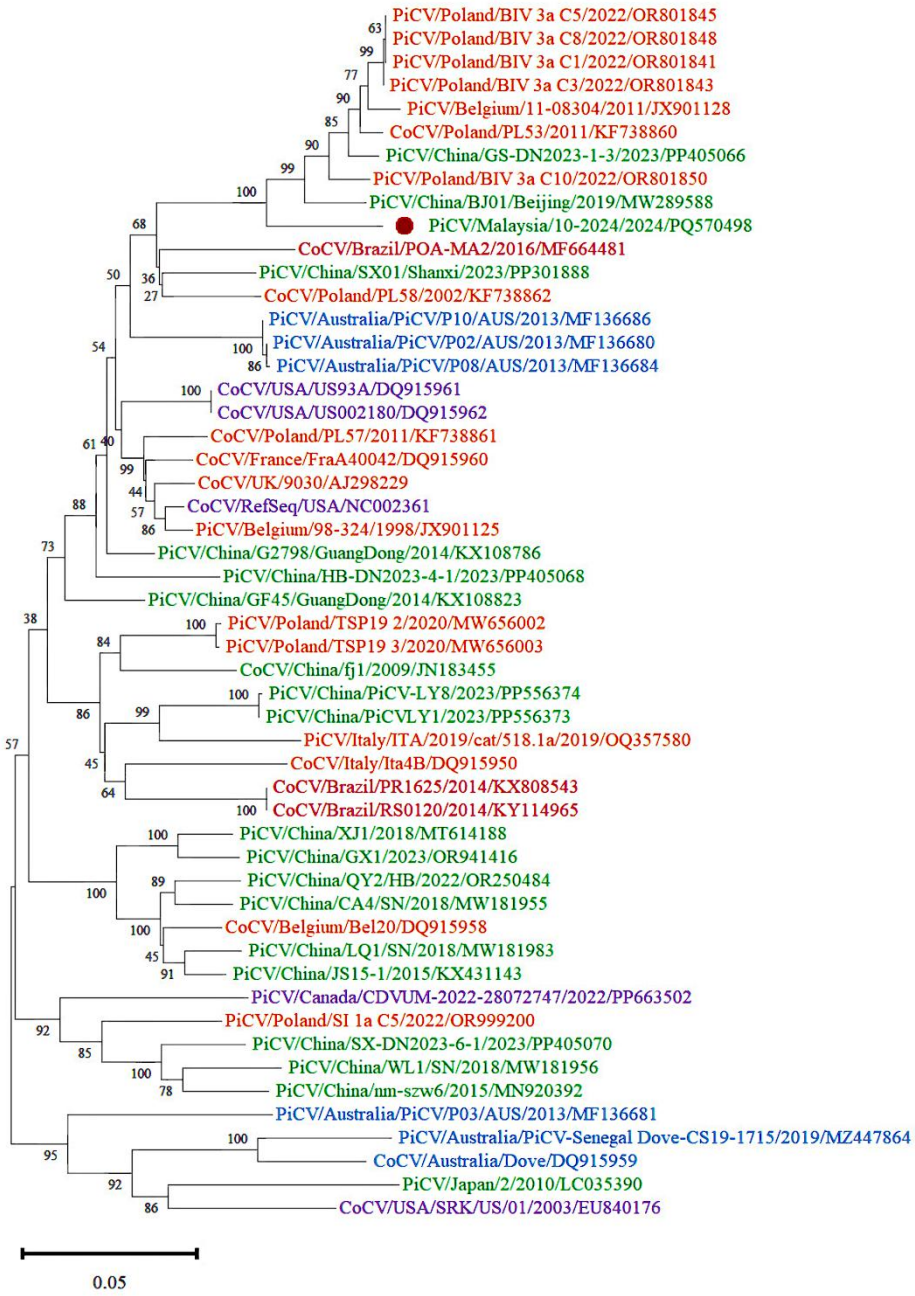


Figure 4.10: Phylogenetic analysis of the full-length pigeon circovirus (PiCV) obtained in the present study, with others 51 full-length PiCV sequences available from the NCBI GenBank database, as listed in Appendix B, Table S3. The phylogenetic trees was constructed using the Neighbor-joining algorithm, with bootstrap test of 1,000 replications in MEGA 11. The PiCV strain obtained in the present study was indicated by a dark red circle (●). To visualize the geographical origins of the PiCV strains, colours were applied to the taxa: orange represents strains from European countries; purple represents strains from North America countries; blue represents strains from Oceania countries; green represents strains from Asian countries; and red represents strains from South America countries. The naming format for each strain was as follows: virus name/origin country/strain/years/accession number in NCBI GenBank.

4.8.2. Rotavirus

The genome of rotavirus consists of 11 segments, with six genes encode for structural proteins and the remaining five genes encode for non-structural proteins. Tables 4.8 and 4.9 show the details of each gene segments for rotavirus A and rotavirus G, respectively, which obtained in the present study. Rotavirus can be classified into nine species group based on the nucleotide sequence of the VP6 gene. The nucleotide identity exceeding 60% in VP6 indicates that the particular strain can be classified into the same species group. From Tables 4.7 and 4.8, the nucleotide identities of VP6 for the rotavirus strains obtained in the present study were 97.17% and 90.20%, respectively, when compared to the highest matching reference sequences of rotavirus A and rotavirus G available in the NCBI GenBank.

The consensus genome of rotavirus A, comprising all 11 segments, was successfully assembled from a total of 18,093 reads (sequences for all 11 segments are listed in Appendix D). According the Rotavirus Classification Working Group (RCWG), the nucleotide identities of each segment must be at least 2% above the cutoff values to assign a species to group A rotavirus. The respective cutoff values for each segment of rotavirus A in the order from VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4 and NSP5 are 83%, 84%, 81%, 80%, 85%, 80%, 79%, 85%, 85%, 85%, and 91%, respectively. In the present study, all segments of rotavirus A achieved these thresholds (Table 4.7), thus, this strain was confidently classified as the group A rotavirus. The assembled

sequences, with total genome length of 18,146 nucleotides for all 11 segments were deposited in NCBI GenBank database, with the respective accession numbers listed in Table 4.9.

By contrast, although rotavirus G was identified as the most abundant eukaryotic RNA virus (80.39% of the viral RNA community), its consensus genome could not be successfully assembled due to the presence of numerous gaps with unidentified nucleotides and ambiguous bases. This suggest that the rotavirus G strain associated among the pigeon flock in UTAR Kampar campus might represent a novel genotype, as it showed low sequence similarity to previously reported strains.

Phylogenetic analysis was performed only for rotavirus A due to the greater completeness of its genome. The rotavirus A sequences obtained in the present study were aligned and compared with seven human strains, six mammalian strains (including virus strains from pig, raccoon, cat, shrew, bovine and fox), as well as 14 avian strains (including *columbidae*, chicken, turkey, gull, velvet scoter, jungle crow and spotted dove), all belonging to group A rotaviruses. The phylogenetic trees for segments encoded for structural proteins are shown in Figure 4.1 to 4.16, while segments encoded for non-structural proteins are shown in Figure 4.17 to 4.21.

Through phylogenetically analysis based on segments VP7 and

VP4 (Figure 4.16 and 4.14, as well as genotype classification using the ViPR-tool, the pigeon rotavirus A strain obtained in the present study was classified under the genotype G18P[17]. This strain exhibited the closest genomic similarity to the GK-684 strain from Germany, which also classifies as the similar genotype. The full genotypes classification for the current strain was G18-P[17]-I4-R4-C4-M4-A4-N4-T4-E(unknown)-H4, corresponding to the segments VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5. It's important to note that the NSP4 gene has a short query sequence length of 506 bp, which limited for genotype identification.

Based on the remaining identified segments, the current strain showed notable genetic distinction from eight pigeon-associated strains, including GK-684, VIC, SX-05, DR-5, PO-13, WVL21015-FL, ARO and K1802315), one avian strain (RK1 from velvet scoter) and one mammalian strain (288356 from red fox) (Figure 4.11 to Figure 4.19, and Figure 4.21). Most of these strains shared the full genotype G18-P[17]-I4-R4-C4-M4-A4-N4-T4-H4, except RK1, which has an unidentified NSP1 gene (genotype A). The sharing of similar genotypes suggesting its pathogenic potential across avian and mammalian species. The current strain shows a distant genetic relationship with human strains (Figure 4.11 to 4.21).

Table 4.7: Viral consensus analysis for rotavirus A.

Virus Protein	Segments	References length of protein (bp)	Length of Query Sequence (bp)	Unidentified nucleotides	Ambiguous bases	Coverage of Query Sequence at Nucleotide Level (%)	Identity Match at Nucleotide Level (%)	Reference Sequence
Structural proteins	VP1	3305	3286	0	0	100	98.63	Rotavirus A (MH568756)
	VP2	2738	2712	9	1	100	98.53	Rotavirus A (MH568757)
	VP3	2559	2566	0	0	99	93.90	Rotavirus A (KT934650)
	VP4	2349	2222	0	0	99	98.87	Rotavirus A (MH568759)
	VP6	1348	1309	0	1	99	97.17	Rotavirus A (MH568761)
	VP7	1065	1029	0	0	100	99.03	Rotavirus A (MH568764)
Non-Structural proteins	NSP1	1870	1847	0	0	99	98.59	Rotavirus A (MH568760)
	NSP2	1043	900	0	0	100	98.56	Rotavirus A (MH568763)
	NSP3	1092	1067	0	0	100	98.13	Rotavirus A (MH568762)
	NSP4	726	506	0	0	99	98.61	Rotavirus A (MH568765)
	NSP5	729	702	0	2	99	98.86	Rotavirus A (MH568766)

Table 4.8: Viral consensus analysis for rotavirus G.

Virus Protein	Virus Protein	References length of protein (bp)	Length of Query Sequence (bp)	Unidentified nucleotides	Ambiguous bases	Coverage of Query Sequence at Nucleotide Level (%)	Identity Match at Nucleotide Level (%)	Reference Sequence
Structural proteins	VP1	3532	3523	564	0	74	89.21	Rotavirus G (MF768264)
	VP2	3002	2737	1027	5	42	88.87	Rotavirus G (MF768265)
	VP3	2335	2343	429	5	81	87.91	Rotavirus G (KC876012)
	VP4	2449	1723	495	3	93	86.29	Rotavirus G (OR800297)
	VP6	1255	1217	150	1	87	90.20	Rotavirus G (OR800299)
	VP7	814	805	0	1	99	92.30	Rotavirus G (MF768269)
Non-Structural proteins	NSP1	1235	1201	153	7	81	90.66	Rotavirus G (OR800300)
	NSP2	1016	996	0	2	99	92.05	Rotavirus G (OR800302)
	NSP3	961	967	0	0	99	88.85	Rotavirus G (MF768261)
	NSP4	627	433	0	0	93	87.44	Rotavirus G (KC876008)
	NSP5	705	707	56	6	89	90.00	Rotavirus G (KC876009)

Table 4.9: NCBI accession numbers of the genome for rotavirus A with 11 segments obtained in the present study.

Virus Protein	Accession number
VP1	PQ570492
VP2	PQ570493
VP3	PQ570494
VP4	PQ570495
VP6	PQ570496
VP7	PQ570497
NSP1	PQ570487
NSP2	PQ570488
NSP3	PQ570489
NSP4	PQ570490
NSP5	PQ570491

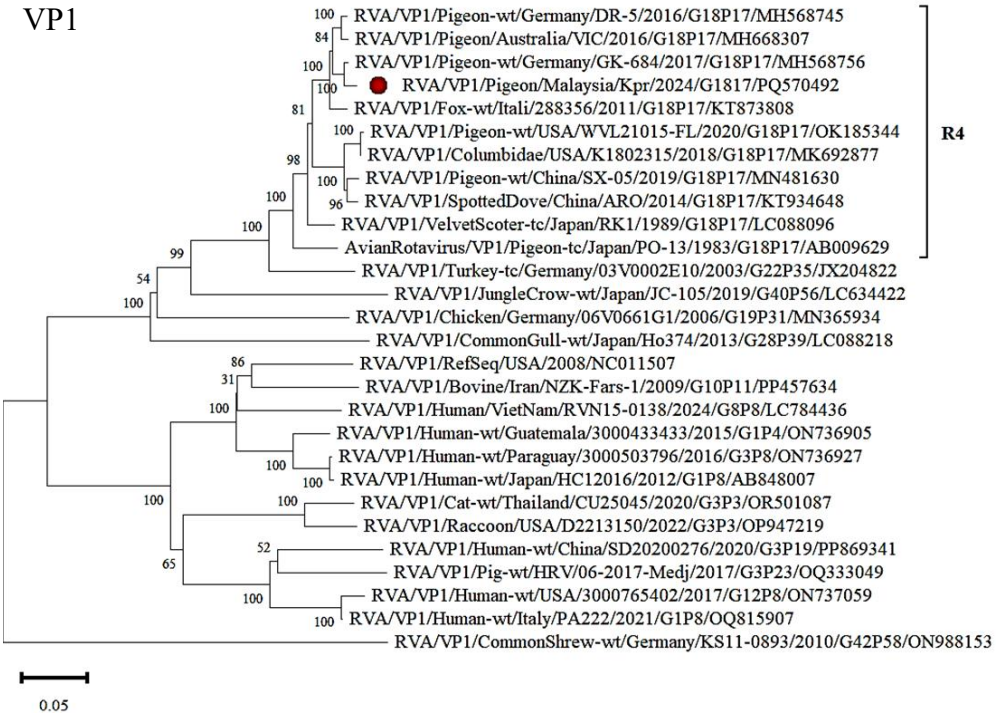


Figure 4.11: Phylogenetic analysis of segment VP1 for rotavirus A, with others 27 rotavirus A sequences available from the NCBI GenBank database, as listed in Appendix B, Table S4. The phylogenetic tree was constructed using the Neighbor-joining algorithm, with bootstrap test of 1,000 replications in MEGA 11. The rotavirus A strain obtained in the present study was indicated by a dark red circle (●). The naming format for each strain was as follows: virus name/gene segment/origin country/strain/years/accession number in NCBI GenBank.

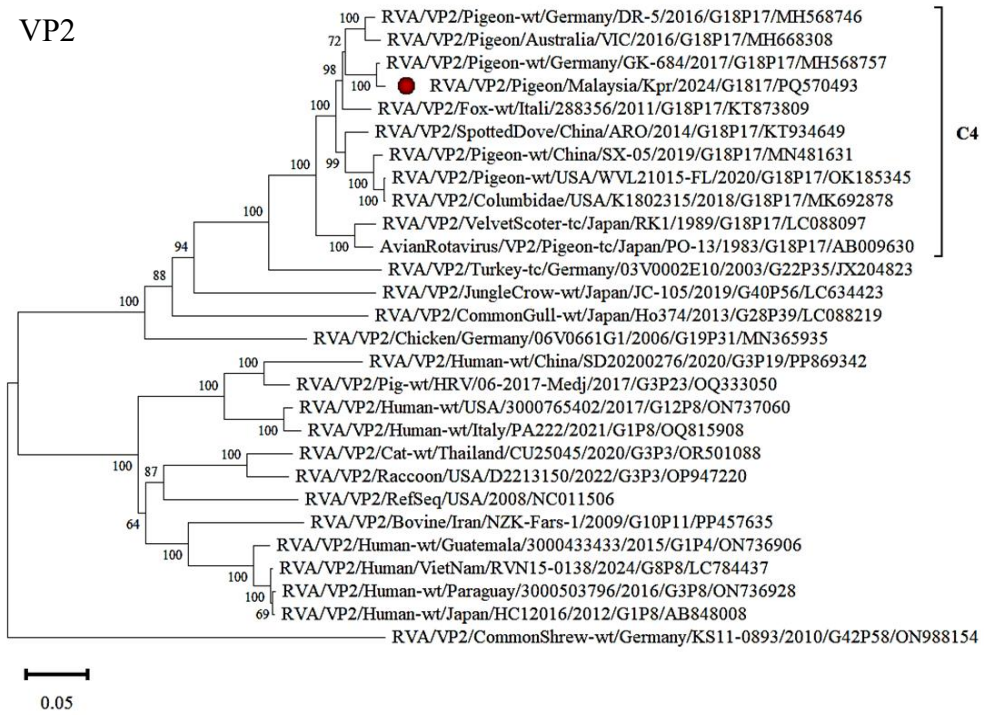


Figure 4.12: Phylogenetic analysis of segment VP2 for rotavirus A. The phylogenetic tree was constructed using the Neighbor-joining algorithm, with bootstrap test of 1,000 replications in MEGA 11. The rotavirus A strain obtained in the present study was indicated by a dark red circle (●).

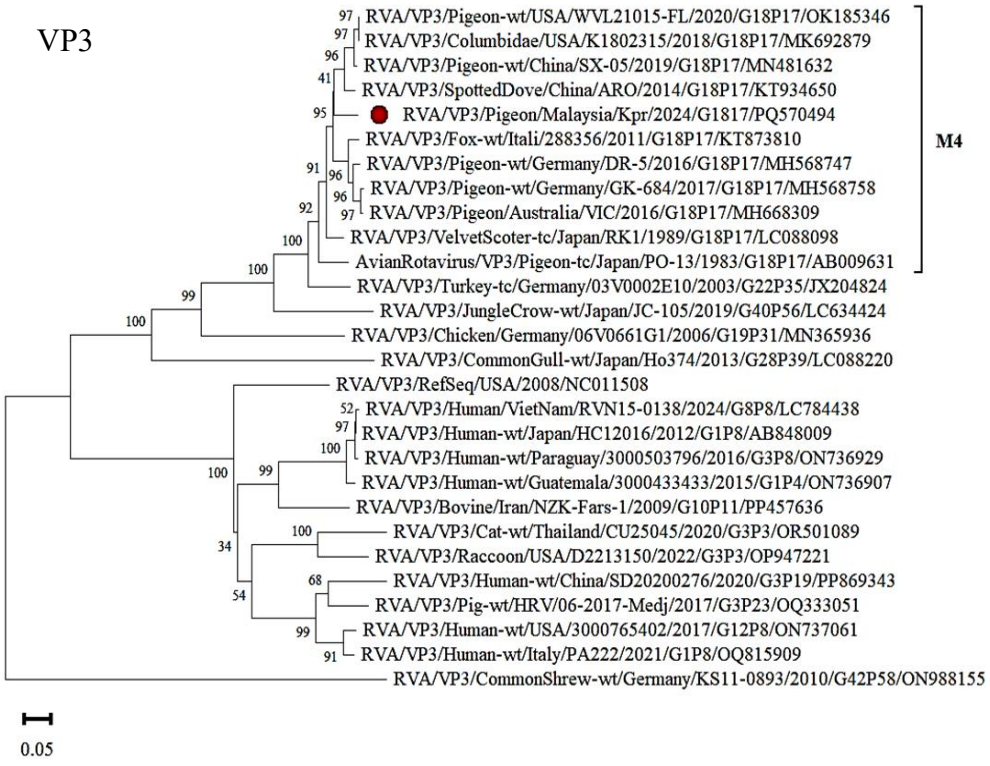


Figure 4.13: Phylogenetic analysis of segment VP3 for rotavirus A. The phylogenetic tree was constructed using the Neighbor-joining algorithm, with bootstrap test of 1,000 replications in MEGA 11. The rotavirus A strain obtained in the present study was indicated by a dark red circle (●).

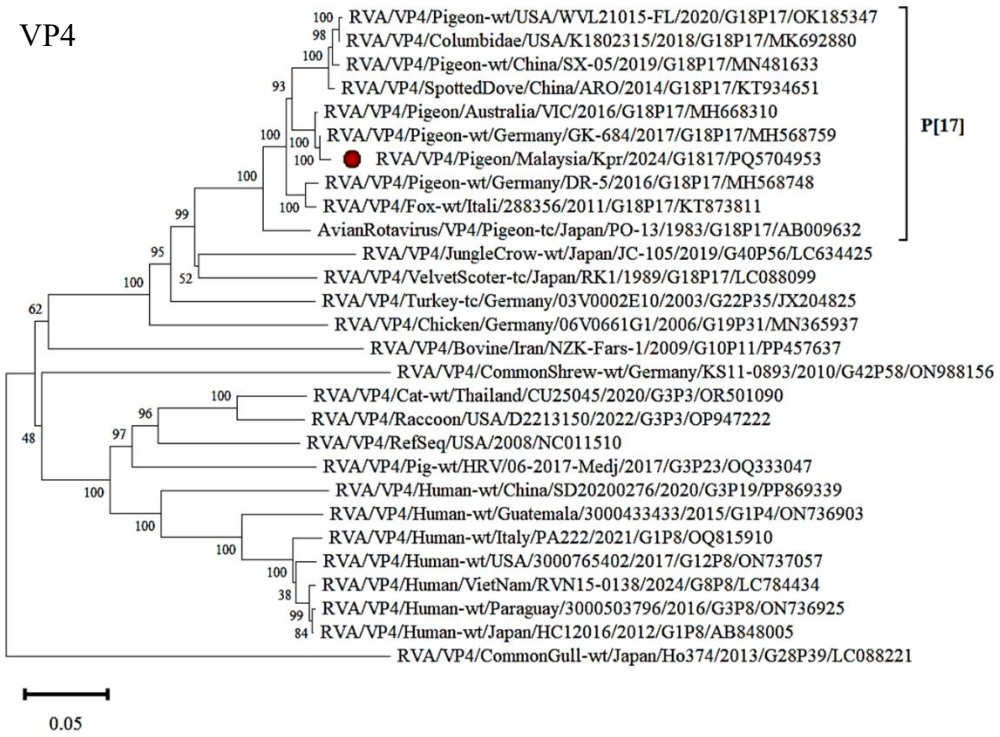


Figure 4.14: Phylogenetic analysis of segment VP4 for rotavirus A. The phylogenetic tree was constructed using the Neighbor-joining algorithm, with bootstrap test of 1,000 replications in MEGA 11. The rotavirus A strain obtained in the present study was indicated by a dark red circle (●).

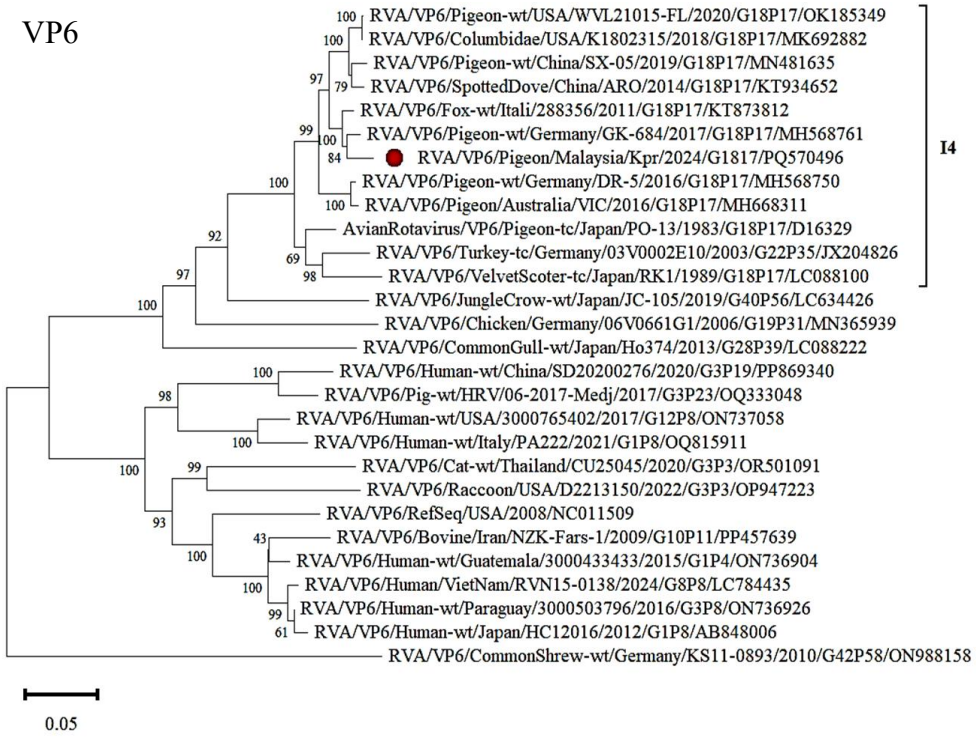


Figure 4.15: Phylogenetic analysis of segment VP6 for rotavirus A. The phylogenetic tree was constructed using the Neighbor-joining algorithm, with bootstrap test of 1,000 replications in MEGA 11. The rotavirus A strain obtained in the present study was indicated by a dark red circle (●).

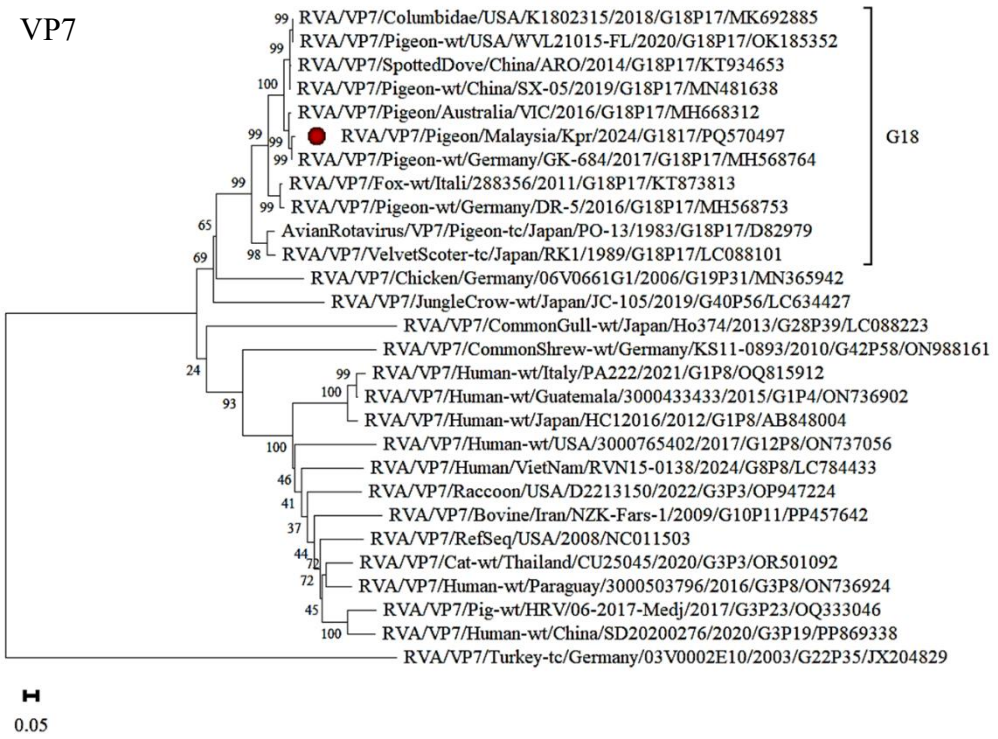


Figure 4.16: Phylogenetic analysis of segment VP7 for rotavirus A. The phylogenetic tree was constructed using the Neighbor-joining algorithm, with bootstrap test of 1,000 replications in MEGA 11. The rotavirus A strain obtained in the present study was indicated by a dark red circle (●).

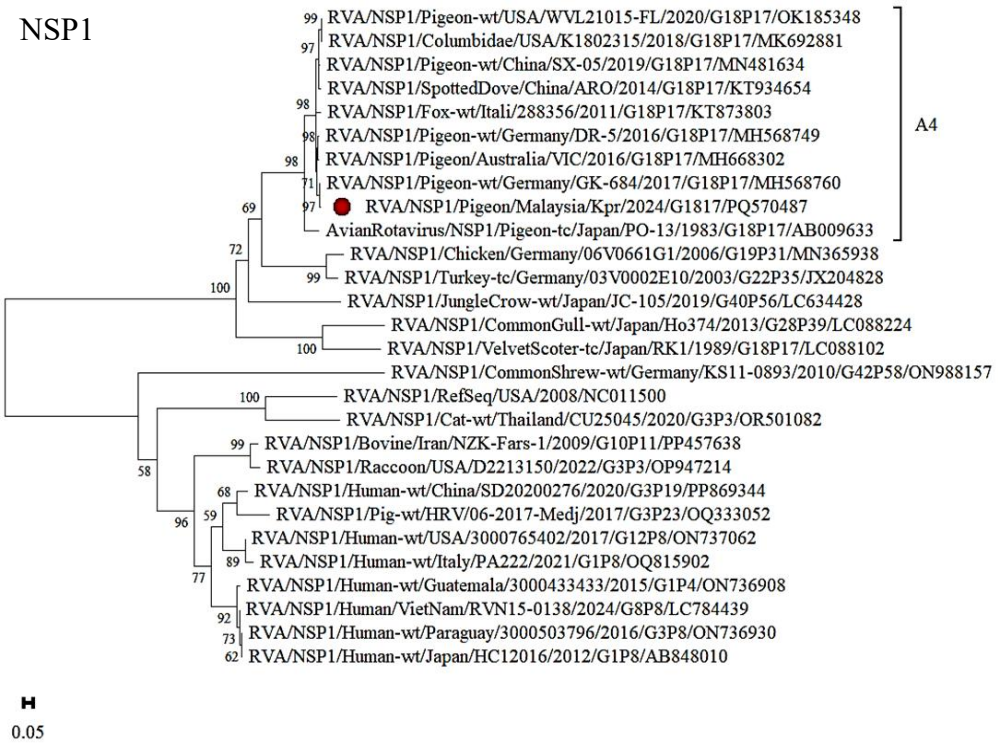


Figure 4.17: Phylogenetic analysis of segment NSP1 for rotavirus A. The phylogenetic tree was constructed using the Neighbor-joining algorithm, with bootstrap test of 1,000 replications in MEGA 11. The rotavirus A strain obtained in the present study was indicated by a dark red circle (●).

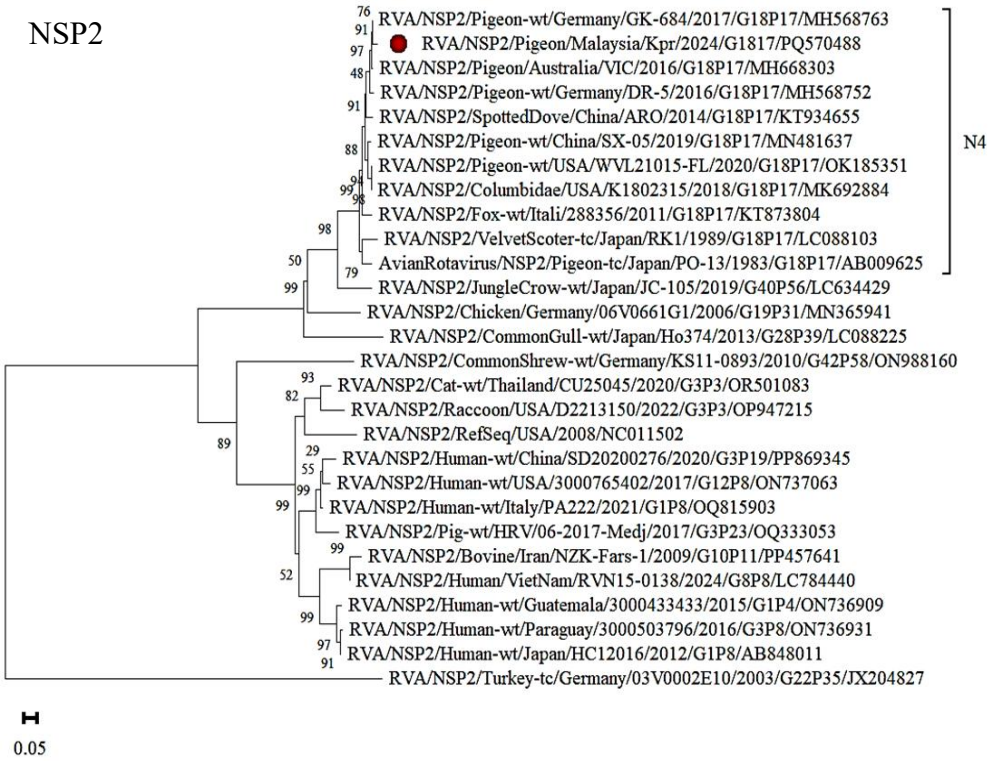


Figure 4.18: Phylogenetic analysis of segment NSP2 for rotavirus A. The phylogenetic tree was constructed using the Neighbor-joining algorithm, with bootstrap test of 1,000 replications in MEGA 11. The rotavirus A strain obtained in the present study was indicated by a dark red circle (●).

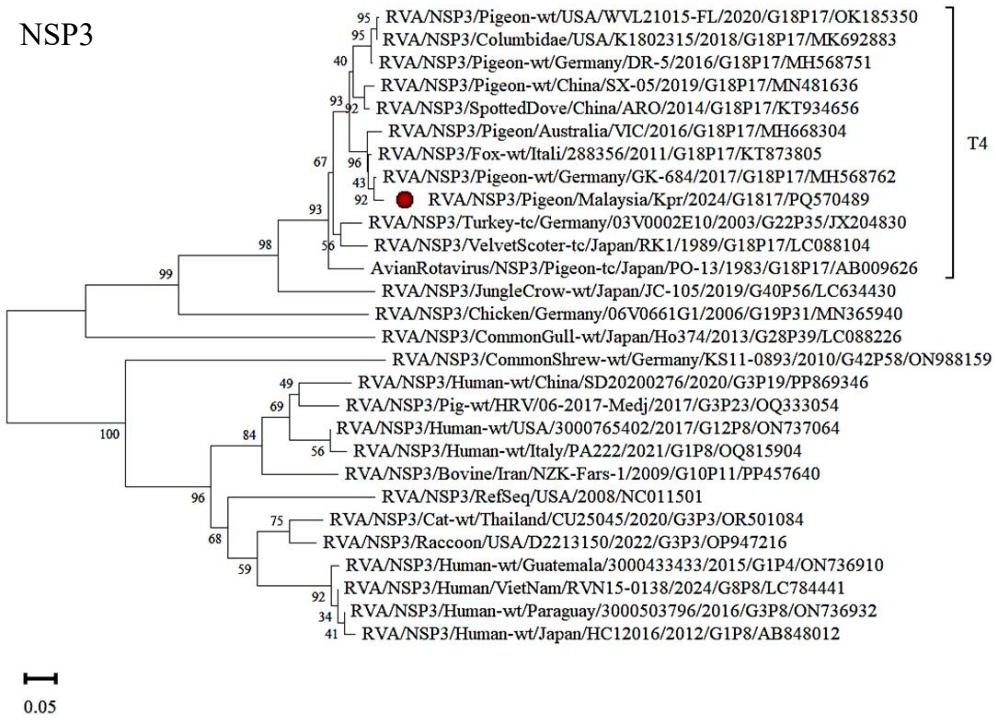


Figure 4.19: Phylogenetic analysis of segment NSP3 for rotavirus A. The phylogenetic tree was constructed using the Neighbor-joining algorithm, with bootstrap test of 1,000 replications in MEGA 11. The rotavirus A strain obtained in the present study was indicated by a dark red circle (●).

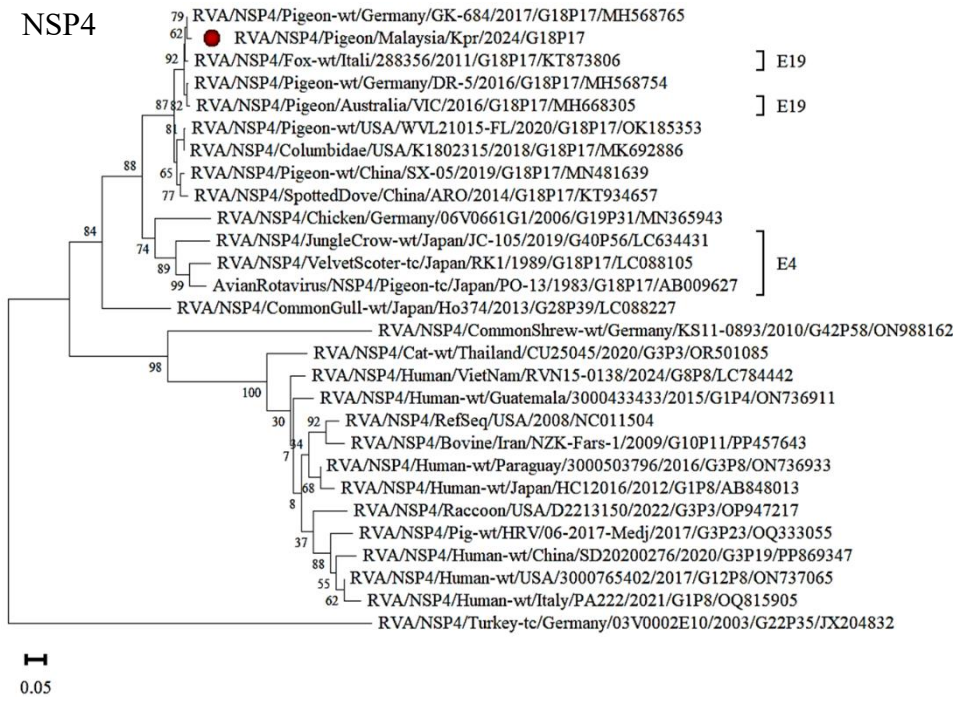


Figure 4.20: Phylogenetic analysis of segment NSP4 for rotavirus A. The phylogenetic tree was constructed using the Neighbor-joining algorithm, with bootstrap test of 1,000 replications in MEGA 11. The rotavirus A strain obtained in the present study was indicated by a dark red circle (●).

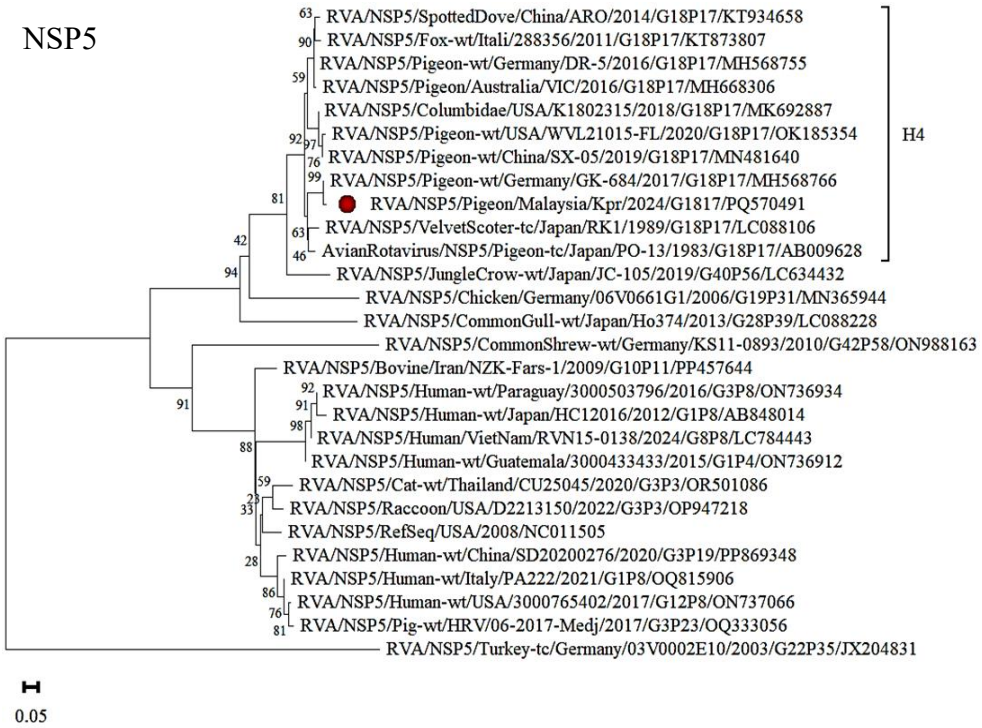


Figure 4.21: Phylogenetic analysis of segment NSP5 for rotavirus A. The phylogenetic tree was constructed using the Neighbor-joining algorithm, with bootstrap test of 1,000 replications in MEGA 11. The rotavirus A strain obtained in the present study was indicated by a dark red circle (●).

CHAPTER 5

DISCUSSION

5.1. Pigeon Dropping Issues in UTAR Kampar Campus

Pigeons are one of the most widespread bird species in urban environments and are frequently observed in close proximity to human populations. UTAR Kampar campus, located in Perak, Malaysia, is situated in a suburban landscape bordered by mountains and lakes, providing a natural habitat for various avian species, including both migratory and non-migratory birds. Pigeons have been observed roosting, resting and nesting in and around campus buildings, such as dining area, hallways, roof edges, and courtyards. This has raised public health concerns, as pigeon droppings are frequently found in areas close to people. For example, droppings have been observed on dining tables, chairs and floors of the cafeterias, near to the water dispensers and along the corridor. Moreover, some pigeons are nesting on the compressor of air conditioners, causing the accumulations of feathers and droppings around the areas, which are seldom cleaned. Thus, one of the purposes of this study is to detect and identify the potential pathogens present in the pigeon droppings, highlighting overlooked hygiene risks and potential health hazards to the UTAR community.

5.2. Composition of Pigeon Faecal Microbiota

This is the first report in Malaysia to investigate the pigeon faecal microbiota across all microbial communities. Bacterial and fungal communities were sorted out from concatenated total DNA genome dataset, comprising an average of 24.5 million paired reads. Eukaryotic DNA viruses were identified from a DNA virus dataset, comprising of 27.6 million paired reads. Eukaryotic RNA viruses were sorted out from concatenated RNA virus dataset, comprising an average of 26.9 million paired reads. Across all three samples, 91.25%, 91.00%, and 89.5% of sequencing reads achieved a quality score of 30 (Q30), meeting the high-quality thresholds, reflecting 99.9% base call accuracy. This study reported that the bacterial community was phylogenetically more diverse compared to fungal and viral communities. Archaea could not be confidently detected due to their extremely low abundance.

Concatenation of two batches of raw sequencing data was performed mainly due to the homogeneity of two batches samples. All droppings were collected under similar environmental conditions and locations, without any significant biological manipulation. The second batch was collected due to the failure of the first batch of DNA virus samples to pass quality control. This is to obtain sufficient coverage and representation of the virus communities. Moreover, the present study involved large-scale sample collection. Pooling of biologically homogeneous samples was purposed to increase the sequencing depth. Thus, support a more robust representation of the overall microbiome

diversity, particularly viral communities, which generally yield lower abundance compare to bacterial and fungal communities, due to their smaller size of genome. The pooling strategy is commonly applied in metagenomic studies when input samples are homogeneous or when targeting low-abundance organisms. Undeniably, this approach limits the detection of rare variants unique to individual samples, however, the primary objective of the present study was to provide a broad overview of the pigeon faecal microbiota.

5.3. Bacterial Community

5.3.1. Bacterial Diversity

In the present study, a total of seven bacterial phyla were detected. The most abundant phyla were *Pseudomonadota* (84.44%) and *Bacillota* (15.26%), together accounting for nearly 99.70% of the bacterial community. The following phyla detected at low proportions included *Actinomycetota* (0.29%) and *Bacteroidota* (~0.01%).

Pseudomonadota comprises gram-negative bacteria. Some members in this phylum being facultative anaerobes. They involve in establishing a gut environment that supports the colonization of obligate anaerobes, such as *Bacillota* and *Bacteroidota*, which are essential for healthy gut function (Shin, Whon and Bae, 2015). In the present study, *Pseudomonadota* was primarily dominated by potential pathogenic genera, including *Escherichia* (78.32%, also was the most predominant

genus in the bacteria community), *Shigella* (1.92%), *Salmonella* (1.39%), and *Klebsiella* (1.06%). The second most abundant phylum, *Bacillota* comprises gram-positive bacteria, are commonly involved in digestion, metabolism of carbohydrates, polysaccharides, fatty acids as well as sugars, and nutrient absorption (Grond, et al., 2018). It also associated with mass gain and immunity in the host (Grond, et al., 2018). In the present study, this phylum was dominated by beneficial probiotic bacterial genera, including *Lactobacillus* (8.74%) and *Limosilactobacillus* (4.41%), which were the second and third most predominant bacterial genera detected in the present study.

Actinomycetota consists of gram-positive bacteria capable of producing hyphae, which inhabiting diverse niches, including the gastrointestinal (GI) tract of vertebrates, soil and aquatic ecosystems (Barka, et al., 2016). *Bifidobacterium*, a genus with probiotic properties, was detected at low abundance (0.03%) within this phylum in the present study. It promotes digestion and inhibits pathogens in the GI system (Servin, et al., 2004; Barka, et al., 2016). Due to its strong adherence to the intestinal epithelium cell, *Bifidobacterium* has been used as a clinical probiotic to prevent the colonization of pathogens (Walsh, et al., 2023). Another phylum, *Bacteroidota* plays a role in fermentation of carbohydrates for energy production, and host immunity (Dietz, et al., 2022; Zafar and Saier, 2021). The metabolic products produced by *Bacteroidota* can reduce the pH, further inhibit the growth of pathogens (Nkosi, et al., 2022; Yan, et al., 2022). Its abundance is influenced by

host diet and metabolism, particularly increase with the intake of high animal-based protein diet (Rinninella, et al., 2019; Dietz, et al., 2022). Based on the observations, the pigeons in UTAR Kampar campus predominantly forage on grass rather than rummaging from the garbage or feeding by humans. Therefore, it can be inferred that the primary diet of the pigeons here is grain- or plant-based instead of animal-based, possible explain the low *Bacteroidota* abundance detected in the present study.

Down to the genus level, the high abundances of *Escherichia*, *Lactobacillus*, and *Limosilactobacillus* detected in the present study was consistent with other previous studies, as these genera constitute a part of the normal gastrointestinal flora of pigeons (Santos, et al., 2020; Khalid, et al., 2023). *Escherichia* has been frequently detected from fresh pigeon droppings in Saudi Arabia (Abulreesh, 2011), Bangladesh (Dey, et al., 2013), and Brazil (Silva, et al., 2009), as well as from pigeon's cloacal swabs and footpads (Dey, et al., 2013). A well-known commensal species, *Escherichia coli*, is involved in digestion, vitamin K synthesis, and the maintenance of anaerobic gut condition (Ingerson-Mahar and Reid, 2011; Martinson and Walk, 2020). However, some *E. coli* strains are pathogenic, and capable for toxin production, leading to gastrointestinal issues. For example, avian pathogenic *E. coli* (APEC) is the main causing agent that causes colibacillosis in birds. The enterohemorrhagic pathogenic strain, *E. coli* O157, which can cause human foodborne illnesses such as bloody diarrhoea, has also been

isolated from pigeon faeces (Wani, et al., 2004; Abulreesh, 2011; Abulreesh, 2014). Despite the high abundance of *E. coli* detected in the present study, its toxicity could not be identified through shotgun metagenomic sequencing. This approach can only infer virulence potential. Importantly, the presence of virulence genes does not confirm the pathogenicity, because the genes fragments might originate from nonviable bacteria, and the gene might not be expressed or might be silenced, depending on gene regulation, environmental stimulations, and host interactions (Enne, et al., 2006; Croxen, et al., 2013; Waseem, et al., 2017; Lema, et al., 2023). Additionally, phylogenetic analyse was not performed on metagenome-assembled genomes of the *E. coli* strains detected in the present study. This was primarily due to the potential presence of multiple *E. coli* strains within the sample. This composite of metagenome-assembled genome from very closely related population can reduce accuracy of data and limit strain-level resolution (Shaiber and Eren, 2019). Moreover, although phylogenetic analyse able to infer the relatedness of *E. coli* strains to known pathogenic lineages, however, it cannot provide the functional insight into virulence gene expression. Alternative methods such as metatranscriptomics and cytotoxicity assays can be applied to evaluate virulence gene expression and pathogenicity (Maldonado, et al., 2005; Chistoserdova, 2009; Lema, et al., 2023).

Lactobacillus and *Limosilactobacillus* are beneficial microbes that exhibit probiotic properties, helping to maintain intestinal health and

produce energy (Xi, et al., 2019; Xu, et al., 2022). These bacteria create an acidic environment through lactose fermentation, which has immunomodulatory effects and allows them to compete with *Escherichia* and other pathogenic bacteria, including *Shigella*, for adherence and replication in the gastrointestinal tract. Thus, protecting the host by inhibiting the colonization of pathogens (Xu, et al., 2022). *Lactobacillus* had been identified as predominant genus in pigeon faeces in Seoul, Korea (Oh, et al., 2023), and has also been found in the crop, small intestine, and rectum of pigeon squabs in China (Ji, et al., 2020).

Overall, the disproportionate dominance of *Pseudomonadota* (84.44%) over *Bacillota* (15.26%), along with low abundance of *Actinomycetota* (0.29%) and *Bacteroidota* (~0.01%), suggests a potential state of microbial imbalance and dysbiosis. *Pseudomonadota* and *Bacillota* are known for antagonistic interaction in avian gut (Grond, et al., 2018; Xu, et al., 2022; Khalid, et al., 2023). In the previous studies, a relative higher abundance of *Bacillota* (ranged from 59.39% to 91.11%) was reported compared to *Pseudomonadota* (ranged from 7.8% to 22.06%) in health pigeon faecal, rectum and cloaca (Grond, et al., 2019; Ji, et al., 2020; Dietz, et al., 2022; Liu, et al., 2022). In contract, *Pseudomonadota* was detected 5.5-fold more abundant than *Bacillota* in the present study. *Actinomycetota* and *Bacteroidota* were also underrepresented relative to previous studies, which reported *Actinomycetota* ranging from 3.86% to 51%, while *Bacteroidota* ranged from 8.2% to 9.56% in pigeon faecal or cloacal samples (Grond, et al.,

2019; Dietz, et al., 2022; Liu, et al., 2022). The predominance of *Pseudomonadota*, particularly *E. coli*, over beneficial probiotic genera, including *Lactobacillus*, *Limosilactobacillus*, and *Bifidobacterium*, may indicate potential host pathology (Shin, Whon and Bae, 2015). The implications of this microbial dysbiosis and potential pathology are further discussed in Section 5.6.

5.3.2. Pathogenic Bacteria

In this study, 5.10% of the bacterial community (comprising 38 species) were potential pathogens. These pathogens cause infections in various human body parts. The most common are enteric infections, urinary tract infection and respiratory infection.

5.3.2.1. Enteric Infection

Enteric infections also known as gastrointestinal disease, are characterized by major symptom, diarrhoea. This symptom was observed during the pigeon dropping collections as mainly droppings were watery. *Salmonella enterica* is the most abundant pathogenic bacteria detected in the present study. Pigeons are known reservoirs for this bacteria (Lawson, et al., 2014; De Oliveira, et al., 2018). It is associated with salmonellosis, a foodborne diarrhoeal illness that can spread to the environment through faecal contamination (Coburn, et al., 2007; Annous and Gurtler, 2012; Andino and Hanning, 2015). This infection is commonly transmitted via the oral-faecal route, such as through ingestion of infected poultry meats and eggs or contaminated

water and food sources.

S. enterica can be further classified into more than 2,600 serovars based on their antigenic presentation (Gal-Mor, Boyle and Grassl, 2014). Among these, the pathogenic strain, *S. enterica* serovar Typhimurium has been detected in pigeon dropping in Egypt (Osman, et al., 2013), Croatia (Vučemilo, et al., 2003), India (Dutta, et al., 2013) as well as in pigeon liver and intestine in Brazil (Rocha-e-Silva, et al., 2014). It is a pigeon-associated strain (Rabsch, et al., 2002). The infected pigeons can shed this pathogen through their faeces, which can further spread the infection among the avian flock and pose human health hazard (Pasmans, et al., 2008; Kaczorek-Łukowska, et al., 2021). It has a broad host range, affecting humans and superior primates (Gal-Mor, Boyle and Grassl, 2014). This serovar causes typhoid fever (also known as enteric fever), which is a life-threatening disease. The present study has limitation to further identify the serovar of *S. enterica*. In the perspective of antibiotic resistance for this serovar, the isolated strains from Egypt showed resistance to lincomycin and streptomycin (Osman, et al., 2013). In India, the isolated strains from pigeon dropping and other tissue samples including liver, intestine, spleen and kidney showed resistance to other antibiotics including ampicillin, tetracycline and nitrofurantoin (Dutta, et al., 2013). In the present study, several antibiotic resistance genes such as *CTX-M-1*, *DHA-1*, *qnrS1*, *dfrA14*, *sul1*, and *sul2* that belong to *S. enterica* were detected, indicating the *S. enterica* from pigeon droppings is resistant to multiple antibiotics

including beta-lactams, fluoroquinolone, diaminopyrimidine and sulfonamide. Among these genes, *qnrS1*, *dfrA14*, *sul1*, and *sul2* are plasmid- or integron-associated genes, which are mobile genetic elements that encourage the proliferation of resistance genes within the bacterial population. More details regarding the AMR gene are listed in the following section, 5.3.3.

Shigella species such as *S. flexneri*, *S. dysenteriae*, *S. boydii* and *S. sonnei* were detected in the present study, which contributes to 1.92% of the bacterial community. These bacteria are usually involved in enteric infection in densely populated regions. These enteric bacteria also can be transmitted to humans through the oral-faecal route via contaminated food and water (Puzari, et al., 2018). For human, *Shigella* spp. cause shigellosis with the symptoms such as watery diarrhoea, vomit, abdominal pain or dysentery (Hale and Keusch, 1996). These pathogens can be invasive on the colonic epithelium to cause inflammation of the colon (Hale and Keusch, 1996). Low infective dose, ranges from 10 to 100 bacteria cells are able to cause infection in humans (Puzari, et al., 2018). In the present study, 18 AMR genes associated with *Shigella* species were detected and listed in Table 4.6. This study is consistent with the observations from Ranjbar and Farahani (2019), which reported that the detected *Shigella* species exhibited antimicrobial resistance to different antibiotic classes including β-lactams, tetracyclines and fluoroquinolones.

Another seven enteric pathogens, such as *Aeromonas hydrophila*, *Campylobacter jejuni*, *Clostridioides difficile*, *Clostridium perfringens*, *Edwardsiella tarda*, *Listeria monocytogenes*, and *Vibrio cholerae* were detected in the present study. Most of the enteric pathogens cause infections through ingestion of faecal contaminated food or water sources. Therefore, prevention of pigeon colonisation especially around dining areas is crucial. In UTAR Kampar campus, pigeons were found nesting above the dining area in the cafeteria, Block C. Their droppings were discovered on dining tables, chairs, and floors. Food sold at the stalls was also exposed to the open air without any protection. This situation posed a health risk to cafeteria users as pigeon droppings carry many pathogens that are transmitted through oral-faecal routes. Flies can act as potential intermediate carriers in this transmission. Fortunately, this situation has been improved after the renovation of ceilings, which effectively prevents pigeons from resting and nesting above the dining areas. However, pigeon dropping issues associated with dining areas still persist in other places such as corridors, common areas in front of learning rooms, and the cafeteria located on the ground floor of the UTAR main library. Pigeon control in these areas needs to be addressed. Public awareness among the UTAR communities about the possible health risks associated with pigeon droppings should be raised to encourage people to avoid eating and drinking in these environments.

5.3.2.2. Urinary Tract Infection

Klebsiella spp., *Enterococcus* spp. and other seven bacteria causing urinary tract infections were detected in the present study. The isolation of *E. faecium* and *E. faecalis* from urban pigeon dropping have been reported in Brazil (da Silva, et al., 2012), from cloacal swabs of pigeon in Poland and Spain (Dolka, et al., 2020) as well as other meat parts of pigeon in Spain (Guerrero-Ramos, et al., 2016; Cordero, et al., 2019). *Enterococcus* is part of microflora that found in pigeon intestines (Baele, et al., 2002). However, it also plays a pathogenic role in human infection including hospital-associated infection (HAI). *E. faecium* and *E. faecalis* are associated with clinical aspects (Mundy, Sahm and Gilmore, 2000; Rice, et al., 2003; Facciolà, et al., 2019). *E. faecium* has been isolated from pigeon droppings that collected around a hospital in Brazil, increasing the risk of hospital-associated infections (Vasconcellos, et al., 2022). The isolated *Enterococcus* from the previous study were reported to exhibit multiple antibiotic resistance including erythromycin, tetracycline, ampicillin and kanamycin (Guerrero-Ramos, et al., 2016; Cordero, et al., 2019). In the present study, the AMR gene such as *APH(3')*-IIIa that is associated with *Enterococcus* was detected. The association with antimicrobial resistance raises a serious health concern, especially the pathogenic strains involved in nosocomial infections.

Although the direct urinary tract infection from pigeon to human is rarely reported, however, the antimicrobial resistance should be paid

attentions because it can be horizontally transmitted within the bacterial community. Moreover, some bacteria can cause multiple types of infections. *Klebsiella* spp. accounted for 1.06% of the bacterial community in the present study. This bacteria can cause infections in multiple sites, including both urinary tract and respiratory tract (Jones, et al., 2010; Van Duin, et al., 2013). Urinary tract infection caused by this genus of bacteria are usually associated with urethral catheterization (Van Duin, et al., 2013). In the clinical setting, *K. pneumoniae* has been detected resistant to carbapenem, which causes serious life-threatening infections (Schwaber, et al., 2008; Borer, et al., 2009). Due to this resistance, the fatal rate was up to 60%, especially for immunocompromised individuals (Hirsch and Tam, 2010; Wu, Zheng and Yao, 2022). In the present study, *marA* was detected, which is responsible for resistance to multiple antibiotics including carbapenem for *K. pneumoniae*. It poses a human health hazard, especially hospital-associated infections.

5.3.2.3. Respiratory Infection

K. pneumoniae is also associated with respiratory infection. In healthcare facilities, it is associated with ventilators (Jones, et al., 2010). Other *Klebsiella* species such as *K. oxytoca* and *K. aerogene*s also cause pneumonia (Podschun and Ullmann, 1998). Both of them are also associated with HAI, especially detected in immunocompromised patients, patients admitted to Intensive Critical Care Units (ICU) and neonatal care units (Ramachandran, 1999; Singh,

Cariappa and Kaur, 2016). They exhibit multiple drug resistances that are commonly administered clinically. The clinically isolated *K. oxytoca* was resistant to antibiotics such as β-lactams, carbapenem, imipenem and amikacin (Singh, Cariappa and Kaur, 2016; Pérez-Vazquez, et al., 2019), while clinically isolated *K. aerogenes* was also resistant to carbapenem (Shen, et al., 2017). In the present study, *Klebsiella* spp. associated multiple antibiotic-resistant genes, including *APH(3')*-IIIa, *CTX-M-1*, *DHA-1*, *qnrS1*, *dfrA14*, *sul1* and *sul2* were detected. Once again, the multiple antibiotic resistance of pathogens is a serious issue, which increases the difficulty of infection treatments.

The respiratory infections that posed by pigeon dropping have been frequently reported. According to Haag-Wackernagel and Moch (2004), 99.4% of disease transmission from pigeons to human was contributed through aerosol transmission of airborne excreta. The most frequently reported respiratory pathogen is *Chlamydia psittaci*. This bacteria was detected from pigeon dropping in Korea (Oh, et al., 2023). It was also been detected with rates of 5% during low-breeding periods (late winter) and 10% during high-breeding periods (spring) in the Netherlands (Heddema, et al., 2006). On June 15, 2024, seven cases of psittacosis were reported in the US this year (National Notifiable Diseases Surveillance System, 2024). *C. psittaci* is an airborne pathogen, which capable of long-term survival outside the host body and has been detected in air samples of pigeon flocks (Dovč, et al., 2016; Szymanska-Czerwinska and Niemczuk, 2016), therefore, it can

be transmitted through dried droppings or respiratory secretions, contaminated food and water, or in a rare case, through bird bites (Harkinezhad, et al., 2009, Vázquez, et al., 2010; CDC, 2022a).

C. psittaci Genotype B was notably associated with pigeons (Heddema, et al., 2006, Sachse, et al., 2008; van Lent, et al., 2012; Burt, Röring and Heijne, 2018). This genotype causes zoonotic human infection, psittacosis (Vázquez, et al., 2010). This infection can be asymptomatic or show mild symptoms such as fever, chills, and dry cough until severe systemic disease such as pneumonia, which highly depends on the individual's immunity. Early diagnosis is crucial for preventing serious infection (Harkinezhad, et al., 2009). Although fatal cases are rare, the risk posed by *C. psittaci* should not be underestimated. According to Harkinezhad and collaborators (2009), 25% of individuals with close contact with parrots and racing pigeons were more frequently infected by *C. psittaci*. Many clinical studies have reported that humans were confirmed diagnosed with psittacosis after having contact with pigeons in different countries such as UK, US and Japan (Henry and Crossley, 1986; Mair-Jenkins, et al., 2018; Fukui, et al., 2021) or having contact with other avians such as cockatiels, chicken, and duck in Taiwan, and China (Cheng, et al., 2013; Dai, et al., 2022; Yao, et al., 2022). Human-to-human transmission of psittacosis was also reported (Henry and Crossley, 1986; Mair-Jenkins, et al., 2018; Yao, et al., 2022).

In UTAR Kampar campus, pigeon flocks are often observed resting on windows and around the compressor of the air conditioners. Their feathers and droppings accumulate in these areas. Inhalation of such substances has been reported to cause airborne infections, particularly hypersensitivity pneumonitis (Reed, Sosman and Barbee, 1965; Cramer, et al., 2016; Shirai, et al., 2017). Inhalation of these substances can trigger the type-III immune complex-mediated hypersensitivity reaction and type-IV lymphocytic reaction to further activate T lymphocytes and macrophages in the alveoli (Bourke, et al., 2001; Morell, et al., 2008). The inflammation response was then triggered, leading to acute, subacute or chronic lung infections (Bourke, et al., 2001; Morell, et al., 2008). Circulation of these airborne infectious agents increases the health risks to humans even though some people do not have direct contact with the pigeon flocks. Pigeon controls and continuous surveillance should be conducted to minimize the aerosol transmission of these pathogenic agents from pigeons to humans.

5.3.3. Bacterial Community and Corresponding Resistome

In the present study, 19 antimicrobial resistance (AMR) genes were identified using shotgun metagenomic sequencing. These genes encode various resistance mechanisms, including efflux pumps, enzymatic degradation, target protection or modification, and regulatory systems. Important to note that, the presence of AMR genes that detected using this method indicates a potential of resistance, it does not confirm

the phenotypic resistance. According to Comprehensive Antibiotic Resistance Database (CARD), most of the detected genes are primarily associated with potential pathogenic gram-negative bacteria, such as *E. coli*, *K. pneumoniae, Shigella* spp.*, Enterobacter* spp., etc. Naturally, gram-negative bacteria exhibit higher antibiotic resistance compared to gram-positive bacteria due to the presence of an outer membrane, lipopolysaccharide (LPS).

Efflux pumps are the major contributors to multidrug resistance by actively expelling antibiotics from bacterial cells. In the present study, AMR genes such as *acrE*, *emrB*, *emrK*, *mdtE*, *mdtG*, *mdtH*, and *mdtN*, were detected, conferring resistance to fluoroquinolone, tetracycline, and nucleoside (Xiong, et al., 2000; Reygaert, 2018; Alav, Bavro and Blair, 2021; Reyes-Fernández, et al., 2021). Some efflux pumps often regulated by transcriptional activator or repressor. Gene such as *marA*, *emrR*, and *H-NS* were detected, can modulate the expression of efflux pump, in response to environmental stress. For example, *marA* gene can activates the AcrAB-TolC efflux pump, conferring resistance to fluoroquinolones, ciprofloxacin, and tetracycline (Piddock, 2006; Vogel and Papenfort, 2006; Kuhlmann, Dalhoff and Zeiler, 2012). According to CARD, *marA* gene has been detected in *K. pneumoniae*. Additionally, e*mrR* gene regulates the emrAB-TolC efflux pump, confers resistance to thiolactomycin (Lomovskaya, et al., 1995; Xiong, et al., 2000; Barroso, et al., 2018), while *H-NS* gene modulates expression in response to environmental stress, including the exposure to antibiotics such as beta-

lactams, aminoglycosides, trimethoprim, sulfonamides and colistin (Hommais, et al., 2001; Nishino and Yamaguchi, 2004; Rodgers, et al., 2021).

AMR genes that involved in two-component regulatory system, such as *cpxA* and *evgA*, were detected. Basically, these systems encoded for sensor kinase and response regulator, enabling adaptive responses to environmental stimuli. *CpxA* gene, as part of the *CpxR/CpxA* system, is associated with resistance to tetracyclines, quinolones, and β-lactams, as well as biofilm formation (Weatherspoon-Griffin, et al., 2014; Padilla-Vaca, Mondragon-Jaimes and Franco, 2017; Sharma, et al., 2017; Tiwari, et al., 2017; Jing, et al., 2021). The formation of biofilm can enhance community-level antimicrobial resistance (Donlan, 2001). E*vgA* gene, as part of the *EvgA/EvgS* system, can activates multiple efflux pump complexes (*emrKY*, *mdtEF*, *tolC*, *acrAB* and *mdfA*), contributing to multidrug resistance, and tolerance to heat and acidic conditions (Kato, et al., 2000; Nishino and Yamaguchi, 2002; Eguchi, et al., 2003; Zhang and Wang, 2023). These systems have been reported in *E. coli* and *Shigella* species (Johnson, et al, 2014; Inada, et al., 2021).

In terms of enzymatic degradation, *DHA-1* and extended-spectrum β-lactamase (ESBL) gene, *CTX-M-1* were detected. These genes encode β-lactamase, which can hydrolyse the β-lactam ring structure of antibiotics, thus reducing the binding to penicillin-binding protein (PBP) (Reygaert, 2018). β-lactam is commonly used in clinical

bacterial infections (Pandey and Cascella, 2019). Wild birds have been reported as reservoirs of ESBL-producing *E. coli* (Alcalá, et al., 2016; Wang, et al., 2017a). A study reported that ESBL-producing *E. coli* strains that carried *bla* $_{\text{CTX-M-1}}$ and *bla* $_{\text{CTX-M-15}}$ resistant genes, were found to have double prevalence in Franklin's gulls (*Leucophaeus pipixcan*) than those in local human populations (Hernandez, et al., 2013). Additionally, nine *E. coli* sequencing types that associated in human infections, were also identified in migratory gulls from central Canada (Hernandez, et al., 2013). This finding highlights the potential of zoonotic transmission of AMR genes between avians and humans, as well as the role of migratory birds in the geographic spread of multidrug-resistant bacteria (Hernandez, et al., 2013).

In the present study, AMR genes that associated with mobile genetic elements were also detected. For instance, *APH*(3')-*IIIa*, *qnrS1* and *sul2* are associated with plasmid (Drlica and Zhao, 1997; Tavío, Jacoby and Hooper, 2014; Krause, et al., 2016; Zaidi, et al., 2024), while *sul1* and *dfrA14* genes are encoded in integron (Rådström, et al., 1991; Miranda, et al., 2016). These genes confer resistance to aminoglycoside (Krause, et al., 2016; Zaidi, et al., 2024), quinolones (Drlica and Zhao, 1997; Tavío, Jacoby and Hooper, 2014), sulfonamide (Rådström, et al., 1991), and trimethoprim (Miranda, et al., 2016). Among them, sulfonamides and quinolones resistant bacteria were frequently reported in pigeon faeces (Radimersky, et al., 2010; Blanco-Peña, et al., 2017). Moreover, quinolones and trimethoprim are commonly used in both

human and veterinary settings (Sköld, 2001; Moulin, et al., 2008; Mazurek-Popczyk and Baldy-Chudzik, 2021). In human, *qnrS1* gene has been detected in 71.4% of hospital- and 36.7% of community-acquired *E, coli* and *K. pneumoniae* isolates in Vietnam (Le, et al., 2015). Plasmid sequencing had confirmed the occurrence of genetic transferring among the nosocomial pathogens, suggesting the concerns of treatment failure due to the increased resistance (Le, et al., 2015). The *dfr* gene has also been detected in *Shigella sonnei*, a foodborne pathogen that cause shigellosis, from human faeces (Miranda, et al., 2016). In veterinary settings, especially for livestock productions, a high prevalence (92%) of *dfr* genes and trimethoprim resistance were detected in *E. coli* strains from pigs, piglets and sows' faeces (Mazurek-Popczyk and Baldy-Chudzik, 2021).

The widespread use of antibiotics in human medicine and livestock production has contributed to the release of antimicrobial residues into the environment through wastewater, livestock run-off, or improper pharmaceutical disposal (Reygaert, 2018; Cao, et al., 2020; ECDC, 2021; Esposito, et al., 2024). These residues impose selective pressure that promotes the multidrug-resistant bacteria (ECDC, 2021; Esposito, et al., 2024). Additionally, AMR genes, especially those encoded in mobile genetic elements such as plasmids and intergons, can facilitates horizontal gene transfer within and across bacterial populations, also driving the emergence of multidrug-resistant bacteria (Rådström, et al., 1991; Sköld, 2001; Sánchez-Osuna, et al., 2019).

Several studies have reported the emergence and dissemination of AMR genes in diverse ecosystems. In Doñana wetlands, sulfonamide, teracycline, and quinolones resistance genes have been detected throughout the local food web, which involving wild birds (including geese, stork, and gull), aquatic species (ceayfish), terrestrial animal (lynxes), and environmental compartments such as wastewater sources (Camacho-Muñoz, et al., 2010; Gonçalves, et al., 2013; Kazakova, et al., 2018; Jarma, et al., 2021; Martín-Vélez, et al., 2021). Similar, during winter in the Dechesas of Spain, crane share close habitats with pigs, which might be exposed to livestock faeces during foraging (Jarma, et al., 2021). Tetracyclines resistant genes, *tetW*, commonly used to treat pig infections, were detected in crane faeces, suggesting potential transmission of AMR genes through faecal contamination (De Briyne, et al., 2014; Jarma, et al., 2021). These findings provided clear evidence that AMR genes can circulate within in wildlife, with potential transmission across species who share a common habitat.

The present study was conducted in UTAR Kampar campus, a suburban area surrounded by mountains and lakes that attract diverse avian species. Both sedentary birds such as common myna (*Acridotheres tristis*), olive-backed sunbird (*Nectarinia jugularis*), and spotted dove (*Streptopelia chinensis*), as well as migratory birds such as Chinese pond heron (*Ardeola bacchus*), wood sandpiper (*Tringa glareola*), and forest wagtail (*Dendronanthus indicus*) have been found within the campus. Although pigeons do not appear to have close nesting sites with other

bird species, however, they were frequently observed sharing water sources, particularly pond water and foraging sites. This ecological overlap raises concerns about potential dissemination of AMR genes and multidrug-resistant bacteria between pigeons and surrounded populations via environmental contact. Additionally, the presence of migratory birds further enabling the long-distance AMR gene transmission. This study which utilize shotgun metagenomic sequencing has limitation in direct attribution of AMR genes to specific bacterial hosts. The detection of AMR gene also do not confirm the phenotypic resistance, as the genes might be silent, or not expressed. This approaches also has limited information about the viability of bacteria. To overcome these limitations, complementary approaches such as culture-based validation, and long-read sequencing can be integrated.

5.4. Fungal Community

5.4.1. Fungal Diversity

Among the three microbial communities analysed, fungi exhibited the lowest phylogenetic diversity. Only four genera, *Kazachstania* (94.11%), *Trichosporon* (3.56%), *Debaryomyces* (1.82%) and *Saccharomyces* (0.51%) were detected in the present study.

The predominant genus, *Kazachstania* (94.11%), belongs to the family, *Saccharomycetaceae*, is commonly found in natural environments such as soil, plants, and fermented foods (Limtong, et al.,

2007; James, et al., 2015). *K. unispora*, also known as *Saccharomyces unisporus* (Bhattacharya, et al., 2013), or *Monosporozyma unispora* (Liu, et al., 2023). It is utilised as non-conventional yeasts in sourdough fermentation (Korcari, et al., 2021). It ferments galactose instead of lactose, thereby avoiding competition for lactose and carbon source with lactose-fermenting bacteria during fermentation process (Montanari, et al., 1996; De Vuyst and Neysens, 2005; Venturi, Guerrini and Vincenzini, 2012). *K. unispora* is able to sustain under stress conditions such as high osmolarity, acidity, ethanol, and salt, as well as exhibits probiotic characteristic and acidic tolerance that enabling survival in the gastrointestinal tract (Goktas, et al., 2021; Korcari, et al., 2021). Another species, *K. bovina*, is pathogenic and is discussed in Section 5.4.2.

The second most abundant genus, *Trichosporon* (3.56%), is a yeast-like fungus commonly detected in natural environments with warm and tropical temperature (Castano, et al., 2023). It has been found in various habitats, including soil, river, fermented dairy products, as well as faeces of pigeons, bats, and cattle (Sugita, et al., 2000; Costa, et al., 2010; Wu, et al., 2012; Castano, et al., 2023; Nualmalang, et al., 2023). Additionally, *Trichosporon* is part of the human normal flora found on skin, vagina, gastrointestinal, and respiratory tract (Castano, et al., 2023; Li, et al., 2020). The only species detected in the present study is *T. asahii*, an opportunistic pathogenic fungus to be discussed in detail in Section 5.4.2. The remaining small portion of fungal community consists of genera *Debaryomyces* (1.82%) and *Saccharomyces* (0.51%).

Both genera had been detected from pigeon dropping in Thailand (Nualmalang, et al., 2023) and China (Wu, et al., 2012). Specifically, the species identified in the present study were *D. hansenii*, *S. uvarum* and *S. kudriavzevii*. None of them were pathogenic.

Compared to previous studies, dominant fungal genera such as *Cryptococcus*, *Candida*, and *Rhodotorula* were frequently detected in pigeon droppings from Brazil (Costa, et al., 2010), Grand Canary Island (Rosario, et al. 2010), China (Wu, et al., 2012), Iran (Soltani, et al., 2013), Saudi Arabia (Abulreesh, et al., 2019), and Thailand (Nualmalang, et al., 2023). However, the present study reported a significantly lower fungal diversity. Three methodological and environmental factors may account for this discrepancy. First, sample freshness and moisture content. In the present study, fresh faecal samples with high moisture contents were processed within 12 hours upon collection and immediately subjected to genome extractions to minimise the possible of environmental contamination. In contrast, previous studies reported that dried droppings, which had been exposed to air for extended periods, promoted the growth of fungi, including *Cryptococcus neoformans* (Wu, et al., 2012; Vallejo, et al., 2016). Lee, et al. (2017) reported that dried droppings supported greater fungal diversity, detecting 35 fungal species compared to 18 species in fresh droppings collected from the same locations. Additionally, pigeon has a body temperature approximately around 40 ºC (Nualmalang, et al., 2023), which is not the optimum temperature for fungal growth. The additional fungal species detected in

dried droppings may reflect contamination from environmental airborne fungal spores, as pigeon droppings contain nutrients such as phosphorus and nitrates that facilitate fungal proliferation (Armstrong, 1984; García-Rowe and Sáiz-Jiménez, 1991; Lee, et al., 2017).

Second, each collected faecal sample was pre-processed by removing the white paste before the nucleic acid extractions. This white paste is rich in uric acid (Spennemann, Pike and Watson, 2017; Critchley, 2021), which can reduce the purity of the extracted genome and subsequently leads to the failure of library construction and sequencing. Due to the anatomical structure of pigeons, their excreta are a mixture of urine and faeces, containing salts, phosphoric acid, nitric acid and uric acids (Spennemann, Pike and Watson, 2017). This acidic condition promotes fungal growth, particularly *Cryptococcus* spp., *Candida* spp. and *Rhodotorula* spp. (Costa, et al., 2010). Previous studies did not mention any pre-processing of collected faecal samples. The exclusion of the acidic component in the present study may have eliminated certain fungal species.

Third, the absence of fungal enrichment steps before genome extraction may have limited fungal detection. Several studies enriched fungi by culturing on the selective media, such as Sabouraud dextrose agar (SDA) with chloramphenicol, to enhance fungal detection (Costa, et al., 2010; Rosario, et al. 2010; Wu, et al., 2012; Soltani, et al., 2013; Medina, et al., 2017; Abulreesh, et al., 2019; Queiroz-Aaltonen, et al.,

2021; Nualmalang, et al., 2023). This culturing process increases the relative abundance of fungi under laboratory setting, thereby improving the sensitivity of the detection. However, it does not reflect the absolute fungal abundance present in pigeon faeces.

Other factors such as diet, climate, environment, and host health status also influence the fungal diversity. Although the present study reported relatively low fungal diversity, the role of dried excreta should not be overlooked. Dried excreta is a major medium for airborne fungal transmission (Naz, et al., 2017). It has been observed in UTAR Kampar campus, particularly around compressor of air-conditioner, window ledges, and structural beams, which are seldom cleaned. The presence of potential pathogenic fungi in pigeon droppings raises potential health concerns, especially in the areas with poor sanitation.

5.4.2. Pathogenic Fungi

In this study, 5.25% of the fungal community (comprising two species) were identified as potential pathogens.

The first species, *Trichosporon asahii*, accounted for 3.56% of the fungal community. A case study reported that a 64-year-old man, who had close contact with pigeons, and his 32-year-old daughter developed summer-type hypersensitivity pneumonitis (SHP). Specific IgA antibodies against *T. asahii* and pigeon dropping extracts were detected in father's bronchoalveolar lavage fluid, suggesting co-exposure

(Makinodan, et al., 2005). In clinical settings, this fungus has been associated with infections linked to invasive medical devices and ICU stays (Kontoyiannis, et al., 2004; Ruan, Chien and Hsueh, 2009).

T. asahii causes trichosporonosis in immunodeficient, immunocompromised, or immunosuppressed individuals, as well as in the patients with hematologic conditions (Krzossok, et al., 2004; Vashishtha, Mittal and Garg, 2012; Li, et al., 2020). It can cause superficial infections, characterized by the formation of irregular nodules on hair, as well as invasive infections such as brain abscess, meningitis, pneumonia, endocarditis and uterine infection (Benson, Lapins and Odom, 1983; Colombo, Padovan and Chaves, 2011; Mehta, et al, 2021). *T. asahii* has been detected in 13% of pigeon excreta in Thailand (Nualmalang, et al., 2023), and Southern Iran (Pakshir, et al., 2019). In Spain, it has also been detected from pigeon cloaca, crop and excreta, with respective abundance of 1.21%, 3.65%, and 9.63% (Medina, et al., 2017).

The second pathogenic species was *Kazachstania bovina*, accounted for 1.69% of the fungal community in the present study. *K. bovina* also known as *Arxiozyma bovina* (Liu, et al., 2023). It has been detected from the nasal passage of pigeons in USA (Kurtzman, et al., 2005). In natural, *K. bovina* can form a fungal complex with distinct species including, *K. telluris, K. pintolopesii*, *K. sloofiae* and *K. heterogenic*, collectively known as *K. telluris* species complex

(Kurtzman, et al., 2005; Kaeuffer, et al., 2022). A case study reported that *Kazachatania* spp. were detected in three patients, aged 63 to 67 years, with underlying health conditions such as diabetes, endometrial cancer, esophagus squamous cell carcinoma, endocrine carcinoma and recurrent angiocholitis (Kaeuffer, et al., 2022). These patients presented with invasive fungal infections, including pyelonephritis, fungaemia, mediastinitis and angiocholitis (Kaeuffer, et al., 2022). Through the MALDI-TOF and sequencing analysis, *K. telluris* complex, including *K. bovina* species, had been identified in two patients with contact history with pigeons (Kaeuffer, et al., 2022). Environmental surveillance further confirmed the presence of *K. bovina* in pigeon dropping from one patient's pigeon aviary, via ITS sequencing (Kaeuffer, et al., 2022). This finding suggested that the underlying health conditions may predispose individuals to *Kazachatania* infection. However, the zoonotic potential and role of pigeons in the transmission of *K. bovina* to humans remain unclear (Kaeuffer, et al., 2022).

5.5. Virus Communities

5.5.1. DNA Viruses Diversity and Pathogens

From the perspective of viruses, two single-stranded DNA viruses, pigeon circovirus (73.33%), belongs to families *Circoviridae*, and pigeon parvovirus A (26.77%), belongs to *Parvoviridae*, were detected in the present study. Both viruses have been detected in pigeon droppings from Hong Kong and Hungary (Phan, et al., 2013). Pigeon

circovirus and pigeon parvovirus A are avian pathogens primarily infecting young birds, leading to weight loss and stunted growth in pigeons.

Pigeon Circovirus (PiCV) has circular single-stranded DNA genome. It has been reported from pigeon flocks worldwide (Coletti, et al., 2000; Mankertz, et al., 2000; Abadie, et al., 2001; Taras, et al., 2003; Todd, et al., 2006; Phan, et al., 2013; Chen, et al., 2021; Wang, et al., 2022; Li, et al., 2024). In Beijing, China, all 40 pigeons from a single breeding farm tested positive for PiCV (Li, et al., 2024). This virus also has been detected in various organs including lung, spleen, liver, cecum and bursa of Fabricius. In northern China, 27.5% of sick racing pigeons and 18.6% of healthy pigeons were tested positive with PiCV (Wang, et al., 2022). The infection rates of meat pigeons in the poultry farm were high, ranging from 19.67% to 75.3% (Zhang, et al., 2015; Wang, et al., 2017b). PiCV infects a wide range of pigeon species, including domestic and feral pigeons, ornamental pigeons, racing pigeons and meat pigeons (Nath, et al., 2023). PiCV is pathogenic to pigeons. It is a contributing agent that causes young pigeon disease syndrome (YPDS). This infection is a multifactorial disease, where PiCV play a crucial role in decreasing the immunity of infected pigeons (Raue, et al., 2005). The pathogenicity of PiCV is discussed in detail in Section 5.6.

Other notable circoviruses pathogenic to birds include chicken anemia virus, psittacine beak and feather disease virus and porcine

circovirus, that affect fowl, psittacine birds and pigs, respectively (Abadie, et al., 2001; Nath, et al., 2023). Besides pigeons, PiCV has been detected in other bird species such as plumed whistling ducks, blue billed ducks and magpies in Australasia (Duchatel, et al., 2006). This previous finding indicated that PiCV has been circulating within the avian population and potentially causes spillover infection in the aberrant hosts (Duchatel, et al., 2006). PiCV is thermostable, surviving temperatures as high as 80°C to 85°C (Duchatel, et al., 2006). Additionally, a study on porcine circovirus showed that this virus is resistant to chloroform and acid, until pH 3 (Allan, et al., 1994). Due to these properties, circovirus can persist in the environments for many years, making it easily transmissible both horizontally and vertically among pigeon flocks (Duchatel, et al., 2006). From genome perspective, the consensus genome of PiCV was assembled in the present study and further compared genetically with various reported PiCV strains from different counties. Details are listed in Section 5.5.1.1.

Another DNA virus, pigeon parvovirus A (26.77%) was detected. It is a non-enveloped virus with single-stranded linear DNA genome. Naturally, members of family *Parvoviridae* exhibit a wide host range, targeting invertebrates (subfamily as *Densovirinae*) and vertebrates including reptiles, birds and humans (subfamily as *Parvovirinae*) (Cotmore, et al., 2019). According to International Committee on Taxonomy of Viruses (ICTV), *Parvovirinae* comprises eight genera and more than 55 virus species (Cotmore, et al., 2019). It can cause enteric

disease syndrome, including gastrointestinal disease in pigeons (Kapgate, et al., 2018; Cotmore, et al., 2019).

In poultry production, the emergency of parvovirus among different avian species has caused economic impacts. Goose parvovirus (GPV) that causes Derzsy's disease can infect both geese and ducks while Muscovy duck parvovirus (MDPV) that infects Muscovy duck only are the two famous waterfowl parvoviruses (Kapgate, et al., 2018; Dong, et al., 2022). They exhibit morbidity and mortality rates of 70% to 100% in three- to four-week-old infected geese and ducks. Furthermore, Turkey (TuPV) and Chicken (ChPV) parvovirus has been frequently detected in poultry farms across many countries such as Hungary, Poland, China, India and the UK (Jager, et al., 2021). These viruses have been found in both healthy and infected birds (Jager, et al., 2021). The infected broilers often develop runting and stunting syndrome (RSS), further exacerbating the economic burden (Kapgate, et al., 2018; Jager, et al., 2021).

From genomic perspective, pigeon-associated parvovirus A strain HK8 was first detected in pigeon droppings in Hong Kong (Phan, et al., 2013). It shared a common ancestry with turkey and chicken parvoviruses, indicating its ability to circulate among different avian species (Phan, et al., 2013; Kapgate, et al., 2018). Parvovirus infection often occurs accompanied by other viruses such as rotavirus and astrovirus (Kapgate, et al., 2018). Both rotaviruses and astrovirus were

also detected in the present study.

The partial genome of the pigeon parvovirus A detected in the present study has been assembled. The genome sequence covers 21.5% of the whole genome when aligned with the reference genome of pigeon parvovirus A, strain HK8 (NCBI Accession number: NC076134). The whole genome sequencing of this virus can be carried out to obtain the complete genome sequence.

5.5.1.1. Viral Consensus Genome for Pigeon Circovirus

In the present study, the viral consensus genome assembled for PiCV was partially complete, with a length of 2035 bp. There is a gap with 21 bp of unknown nucleotide, "N" found in the *rep* gene, likely due to low sequence identity with existing reference strains, suggesting the presence of a novel variant of *rep* gene. Fortunately, the gene encoded for *cap* protein is complete, with 819 bp in length. The conserved nanonucleotide motif, 5'-TAGTATTAC-3', located at the apex of the stem-loop structure that acts as the origin of replication (*ori*) of PiCV was detected. Moreover, the four forward repeat hexamers, 5'-GGAGCC-3' were also detected. From the phylogenetic trees constructed using the full-length genome (Figure 4.10), the strain detected in the present study has the closest genetic relationship with the China PiCV strain, BJ01.

The PiCV strain detected in the present study is genetically

similar with the other strains from Asian countries such as China (strains BJ01 and GS-DN2023-1-3) and European countries such as Poland, (strains BIV 3a C10, BIV 3a C3, BIV 3a C1, BIV 3a C18, and PL53), and Belgium (strain 11-08304). From the phylogenetic tree, all strains were found to cluster in a monophyletic clade, with bootstrap support values larger than 77% and short branch length (Figure 4.10), suggesting these strains share a common ancestor and form a globally distributed lineage of PiCV. This finding aligns with previous research findings that indicated PiCV is globally distributed but does not have differences among the geographical distribution (Zhang, et al., 2015). The full-length sequencing can be carried out in the future to obtain the complete genome of the current PiCV strain.

The global distribution of PiCV is related to its transmission pathway. PiCV can easily spread globally within the pigeon population, likely due to pigeon racing activities, bird exhibitions and the import or export of pigeons as breeding stock to maintain the good pedigrees (Duchatel, et al., 2006; Duchatel and Szeleszczuk, 2011; Stenzel and Pestka, 2014; Stenzel and Koncicki, 2017). Consequently, it can be transmitted either horizontally, through faecal contaminated food or air sources via ingestion or inhalation (Stenzel and Koncicki, 2017). This virus can also be transmitted vertically, as it has been detected in the embryo tissues, testes and ovaries of adult racing pigeons, indicating transmission from parents to newly hatched pigeons (Duchatel, et al., 2006). These various possible transmission ways may have resulted in

a high prevalence (73.23%) of PiCV detected from the pigeon flocks at the UTAR Kampar campus.

5.5.2. RNA Viruses Diversity and Pathogens

RNA viruses exhibit greater diversity compared to DNA viruses. The dominant genus detected was rotaviruses (80.43%), consisting of three species: group A, D and G. Rotavirus A is a human pathogen, whereas rotavirus D and G are avian pathogens. Other avian viruses such as orthoreovirus (5.85%), Megrivirus (0.25%) and avian astrovirus (0.02%) were also detected. Additionally, viruses of plants, fungi, insects and ticks belong to families *Tombusviridae* (12.96%), *Partitiviridae* (0.46%), *Polycipiviridae* (0.01%), *Totiviridae* (0.01%) and Nodaviridae (~0.01%) were detected. The presence of plant and insect viruses may originate from food sources. Furthermore, fungal viruses and tick viruses were likely originated from fungi and ticks that carried along with pigeons.

The predominant RNA virus, rotaviruses (80.43%) are non-enveloped viruses, with a double-stranded RNA genome of 11 segments (Matthijnssens, et al., 2022). Rotavirus A has a broad host range infecting both humans and avians, while rotaviruses D and G infect only avian. The first detection of rotavirus A and rotavirus G from pigeon droppings were reported in 1983 and 2013, respectively (Minamoto, et al., 1988; Phan, et al., 2013). Rotavirus A and rotavirus D were also detected in pigeon from Japan and Nigeria (Minamoto, et al., 1988;

Pauly, et al., 2017). This indicates that similar host species can be infected by different and multiple species of rotaviruses (Phan, et al., 2013). Result from the present study is consistent with Phan's perspective, where group A, D and G rotaviruses co-exist in the pigeon flocks in UTAR Kampar campus.

Rotavirus infections are associated with the gastrointestinal tract (Harzer, et al., 2021). This infection is self-limiting but can be fatal due to dehydration, especially for the young avians. However, pigeon-associated rotavirus A strain, with genotype, G18P[17], was reported to cause outbreaks in pigeon flocks with high mortality rate (>45%) (McCowan, et al., 2018; Blakey, et al., 2019; Hunnam, et al., 2019; Rubbenstroth, et al., 2019; Meßmer, et al., 2022). It has caused outbreak among the pigeon flocks in Asian, Europe, US and Australia (Ito, et al., 2001; McCowan, et al., 2018; Blakey, et al., 2019; Hunnam, et al., 2019; Rubbenstroth, et al., 2019; Rubbenstroth, et al., 2020; Schmidt, et al., 2021; Meßmer, et al., 2022). This virus caused asymptomatic infections as well as systemic infections, which had been detected in pigeon liver tissue, leading to hepatic necrosis (Hunnam, et al., 2019; McCowan, et al 2018, Rubbenstroth, et al., 2019; Rubbenstroth, et al., 2020). Pigeon-associated rotavirus A also caused YPDS-like disease, with symptoms such as diarrhea, vomit, anorexia, weight loss and regurgitation in infected pigeons, accompanied with pigeon circovirus (Rubbenstroth, et al., 2020).

In human, rotavirus A is a major cause of seasonal endemic severe acute gastroenteritis, especially in young children under the age of five (Bishop, et al., 1973). It is transmitted through the faecal-oral route, including fomites (Martella, et al., 2010; Matthijnssens, et al., 2022). A study showed that rotavirus was detected through PCR from fomite samples, particularly the one in moisture environments such as toilet and fountain in the day-care centers (Butz, et al., 1993). Additionally, rotavirus was also detected from air samples in a hospital ward where infected children were being treated, suggesting the possibility of aerosol transmission (Dennehy, et al., 1998). It was proven by the detection of antigens in both the pulmonary and gastrointestinal tracts after exposure to the infected respiratory droplets in a mouse model. The mouse model subsequently showed gastrointestinal disease (Prince, et al., 1986).

Once rotavirus A enters the host body, it targets the villi of mature enterocytes in the small intestine, causing vacuolization followed by crypt hyperplasia (Desselberger, 2014). Beyond localized infection in GI tract, systemic infections also have been observed in children and animal models, by affecting the liver, central nervous system (CNS) and respiratory system, as well as causing antigenemia and viremia (Ramig, 2017). For immunocompromised individuals, rotavirus can even cause biliary atresia and pancreas inflammation (Desselberger, 2014).

In Malaysia, rotavirus is responsible for causing 22% to 50% of

all cases of diarrhea in children (Myhealty Ministry of Health Malaysia, 2024). Therefore, vaccination of young children is crucial for protecting them against rotavirus infection. Vaccination has statistically reduced the incidence of infection, hospitalization and deaths among children (Juliao, et al., 2021; Bose, Borrow and Arkwright, 2024; Japa, et al., 2024). In Malaysia, vaccination has successfully prevented 85% to 98% of severe rotavirus cases (Myhealty Ministry of Health Malaysia, 2024).

Globally, serotypes G1 to G4 of rotavirus A are responsible for approximately 90% human infections (Gentsch, et al., 2005). The diversity of rotavirus is contributed by the genetic reassortments, interspecies transmission, points mutation, as well as genetic recombination (Rohwedder, et al., 1995; Ramig, 1997; Mueller and Johne, 2007). The inter-species transmission through animal-human rotavirus reassortment has been reported. For example, the common P and G serotypes including G3, G4 and P1A[8], have been detected in both human and pig (Gentsch, et al., 2005). Moreover, human rotavirus A, strain B4106 with genotype, G3P[14] was hypothesized to have originated from rabbit and subsequently transmitted to humans (De Leener, et al., 2004; Matthijnssens, et al., 2006).

The high degree of genetic diversity of rotavirus increases the importance to construct the full-length genome of rotavirus in order to study the genetic evolution and the conformation of reassortment event. In the present study, consensus genome for rotavirus A was constructed

to further investigate the genomic relatedness between human- and pigeon-associated rotaviruses, and is detailed in Section 5.5.2.1. Whole genome sequencing for all genome segments of rotavirus G can be conducted in future to obtain the complete sequences.

The second dominant avian RNA virus detected in the present study is orthoreovirus (5.85%). This is a non-enveloped virus with a linear, segmented double-stranded RNA (dsRNA) genome (Matthijnssens, et al., 2022). Orthoreovirus targets vertebrates as its natural hosts and transmitted through respiratory droplets and faecal-oral routes (ICTV, 2024). In the present study, two species, avian orthoreovirus and Cataraqui virus were detected.

Avian orthoreovirus has been detected in chicken, turkey, geese and Muscovy duck (ICTV, 2024). It is an avian pathogen that caused chronic respiratory disease for chicken (Fahey and Crawley, 1954). Catararqui virus is an orthoreovirus detected in birds (Wille, et al., 2021). According to Wille and collaborators (2021), two complete genome strains of Cataraqui virus were detected from juvenile and adolescent birds. Low abundance of this virus was also detected from adult birds with age large than three. This suggests that the virus can be transmitted among birds, with lower infection rates in adult birds (Wille, et al., 2021).

Orthoreovirus has broad host range, including mammals. Mammalian orthoreovirus (MRV) has been detected from healthy

human with asymptomatic infection (Sabin, 1959). Infections associated with MRVs are rare but cause mild upper respiratory tract infection for infants and children (Fauquet, et al., 2005; Guglielmi, et al., 2006). MRV has wide geographic distribution (Guglielmi, et al., 2006). In 2007, a human strain named Melaka virus (MelV) was detected from a 39-year-old male patient with acute upper respiratory disease in Malaysia (Chua, et al., 2007). MelV has been transmitted among the family members of the patient as proven by serological evidence (Chua, et al., 2007). The origin of MelV might come from bats, where the isolated MelV shown a close genetic relationship to a fruit bats' reovirus, named Pulau virus (PulV), which was isolated in 1999, from Tioman Island in Malaysia (Chua, 2003; Pritchard, et al., 2006). Another strain that has originated from bat is known as Nelson Bay virus (NBV), which was detected from fruit bats in Australia (Gard and Marshall, 1973).

Interestingly, in the current study area (Kampar), a novel orthoreovirus strain named Kampar virus (KamV), was isolated from a 54-year-old, male patient with symptoms including fever and acute respiratory infection (Chua, et al., 2008). KamV has close genetic relationship with NBV. In this clinical case, the virus's origin remains unknown, but it is suspected to be a bat-borne virus due to the genetic similarities with the bat-borne orthoreovirus such as MelV, PulV and NBV (Chua, et al., 2008). Additionally, the house of the patient was close to the area where the fruit bats obtained their food (Chua, et al., 2008). Human-to-human transmission was also detected in the Kampar

case. The patient's doctor, a 28-year-old female, developed symptoms such as mild sore-throat and tested positive with antibodies against KamV (Chua, et al., 2008).

In the present study, two orthoreovirus species have been detected and contributing 5.85% to the RNA virus community. Further serological and virological surveillance tests are recommended to be conducted between KamV and orthoreovirus from pigeon, in order to further ascertain whether zoonotic transmission of this virus between humans and pigeon is occurring.

The third dominant avian RNA virus detected in the present study was Megriviurs (0.25%). This virus belongs to family *Picornaviridae*. Members under this family are non-enveloped viruses, with a linear positive-sense, single-stranded RNA genome (Zell, et al., 2017; Łukaszuk and Stenzel, 2020). They have been found in faecal and respiratory specimens from mammals including humans, birds, fishes, reptiles and amphibians (Zell, et al., 2017). There are more than 30 genera under this family (Zell, et al., 2017). In the study from Phan and collaborators (2013), two novel pigeon picornaviruses, Mesivirus-1 and Mesivirus-2, were identified in Hong Kong and Hungary. These two strains show the closest identity to the turkey hepatitis virus, under the genus Megrivirus, reflecting a wide distribution of the viral clade (Phan, et al., 2013). According to NCBI taxonomy browser, megrivirus B consists of two subspecies; megrivirus B1, which is also known as

pigeon mesivirus 1 or picornavirus HK21, and megrivirus B2, which is known as pigeon mesivirus 2.

Nine DNA and RNA viruses detected in the present study are avian viruses. Only one human pathogenic virus, rotavirus A was detected. The natural environment of UTAR Kampar campus attracted many birds, including both migratory and non-migratory species. The sharing of common environmental resources such as water and foraging sites among the birds could be a possible source for virus circulation among the existing avian populations in this campus. Long-term and continuous surveillance of virus circulation among the avian population in this campus is recommended, especially for zoonotic viruses such as rotavirus.

5.5.2.1. Viral Consensus Genome for Rotavirus A

Based on double nomenclature classification, the genotype of the rotavirus A strain detected in the present study is G18P[17]. This genotype has been epidemiologically associated with pigeons in Australia, Germany, Japan, Italy and U.S., having a morbidity rate up to 100% and a mortality rate ranging from none to more than 45% among the pigeon flocks (Ito, et al., 2001; McCowan, et al., 2018; Blakey, et al., 2019; Hunnam, et al., 2019; Rubbenstroth, et al., 2019; Rubbenstroth, et al., 2020; Schmidt, et al., 2021; Meßmer, et al., 2022).

Rotavirus A strains DR-5 and DR-7, isolated from homing and

racing pigeons in Germany with a similar serotype, G18P[17] has been proven to cause young pigeon disease syndrome (YPDS)-like infection in healthy juvenile pigeons (Rubbenstroth, et al., 2020). These strains caused mortality rates of 2.5% and 12.5%, respectively, in the pigeon flocks (Rubbenstroth, et al., 2019; Rubbenstroth, et al., 2020). Both virus strains induced a 100% morbidity rate when inoculated into pigeons as an animal model. Infected pigeons showed acute infection with symptoms such as diarrhea, vomiting, anorexia and weight loss within two to three days after virus inoculation. Viral shedding was also detected in pigeon faeces as well as cloacal up to one-week post-infection. After 21 days, necropsy analysis of the infected pigeons showed mild to severe hyperplasia of spleen. These studies confirmed that pigeon-associated rotavirus A, with genotype G18P[17] acts as a primary pathogen causing YPDS-like disease (Rubbenstroth, et al., 2020).

Additionally, the full genotype classification for the rotavirus strain obtained in the present study was G18-P[17]-I4-R4-C4-M4-A4-N4-T4-E(unknown)-H4, from Figure 4.11 to Figure 4.21 (strain detected in the present study was indicated by a dark red circle). The phylogenetic analysis of the rotavirus A strain from the present study and other eight pigeon-associated strains (GK-648, VIC, SX-05, DR-5, PO-13, WVL21015-FL, ARO and K1802315) showed a close genetic relationship. Moreover, the current Kampar strain also showed a close genetic relationship with other avian strain (RK1 from velvet

scoter) as well as mammalian strain (288356 from red fox). All of these strains share a similar genotype, G18-P[17]-I4-R4-C4-M4-A4-N4-T4-H4, except RK1, which has an unidentified NSP1 gene (genotype A).

In the present study, genotype E, which corresponds to the NSP4 gene was unidentified due its short length of the assembled query sequence (Figure 4.20). NSP4 protein exhibits enterotoxin activity that triggers diarrhea (Lorrot and Vasseur, 2007). Only two pigeon-associated NSP4 (genotype E) variants have been identified. The first one is genotype E19, detected from rotavirus A strain VIC from infected racing pigeons in Australia that exhibited vomiting and diarrhea (McCowan, et al., 2018). Genotype E19 was first reported from an infected red fox with encephalitis, where the rotavirus A virus was isolated from its brain cells (Busi, et al., 2017). This genotype was then detected in various avian species in bird markets and farms, including pigeons, chickens, ducks, guinea fowls and quails in Nigeria (Pauly, et al., 2017). Inter-species transmission of rotavirus A has been detected (Pauly, et al., 2017). Infected pigeons in Nigeria showed clinical symptoms such as diarrhea, vomit and hepatic necrosis (Pauly, et al., 2017). The detection of genotype E19 in mammalian and various avian species reflects the potential cross-species transmission, especially among avian hosts who are sharing the same living spaces. However, the exact mechanisms of this cross-species transmission remain unclear (McCowan, et al., 2018).

The second genotype E4 is associated with the strain PO-13, which detected from a feral pigeon in Japan (Minamoto, et al., 1988; Ito, et al., 2001). The PO-13 strain isolated from pigeon faecal sample was the first full-length avian rotavirus (Minamoto, et al., 1988). This strain was cultured in mammalian cell lines, including fetal rhesus monkey kidney (MA-104) and bovine kidney (MDBK). It subsequently infected both cell lines *in vitro*, which induced cytophatic effect (Minamoto, et al., 1988). Additionally, experimental studies carried by Mori and collaborators (2001) successfully demonstrated that PO-13 caused infection in a mouse model (Mori, et al., 2001). In that experiment, 3-day-old suckling mice were orally introduced with PO-13 and developed diarrhea within one to two days post-inoculation (Mori, et al., 2001). This finding highlighted the pathogenic potential of this avian rotavirus in a mammalian host system. Importantly, the infection rate in mice was age-dependent, with a rapid decrease in mice older than five days (Mori, et al., 2001).

In short, these findings highlight the pathogenicity of pigeon-associated rotavirus A, especially the genotype G18P[17] and its ability to infect both avian and mammalian models. Full-length sequencing of all viral genome segments, especially the NSP4 gene, is recommended to further study the cross-species transmission and reassortment event of rotavirus. Long-term surveillance study is also recommended for viral evolutionary study, in order to further investigate whether this virus strain may be zoonotically transmitted to humans.

5.6. Co-occurrence of Potential Pathogenic Bacteria and Viruses, and Their Implications for Host Health

At the phylum level of bacterial community, *Pseudomonadota* (84.44%) was the most dominant phylum in the present study, significantly exceeding *Bacillota* (15.26%) by 5.5-fold. Both phyla are commonly found in avian GI tract, with an antagonistic interaction (Grond, et al., 2018; Xu, et al., 2022). In healthy pigeon droppings and rectums, *Bacillota* was detected at higher abundance (ranging from 59.39% to 91.11%) compared to *Pseudomonadota* (ranging from 7.8% to 22.06%) (Ji, et al., 2020: Liu, et al., 2022). In contrast, the reversed distribution was reported in unhealthy avians, where an overrepresentation of *Pseudomonadota*, especially *E. coli* had often been reported. In broilers with colibacillosis, a threefold increase in *E. coli*, accompanied by a fourfold decrease in *Lactobacillus* have been observed in the ileum (Khalid, et al., 2023). Colibacillosis is an enteric disease associated with diarrhea and sluggish growth (Adil, 2020; Khalid, et al., 2023). Similar microbial distributions have also been reported in the droppings of pigeons with enteric disease and diarrhoea (Fan, et al., 2024), and small intestine and rectum of pigeon infected with parasite, *Trichomonas* gallinae (Ji, et al., 2020). Therefore, the predominance of *Pseudomonadota* suggests potential pathogenicity (Shin, et al., 2015). In the present study, *E. coli* accounted for 76.41% of the bacterial genera, exceeding *Lactobacillus* (8.74%) by 8.7-fold. Although both are part of the normal gut flora, this imbalance suggests microbial dysbiosis, likely

caused by the overgrowth of *E. coli* outcompeting commensal and probiotic bacteria.

This dysbiosis might be attributed to co-infection with pigeon circovirus (PiCV). In the present study, PiCV was predominant in the eukaryotic DNA virus community (73.23%). This virus is associated with young pigeon disease syndrome (YPDS), a multifactorial disease characterized by immunosuppression through B lymphocyte apoptosis in lymphoid organs (Abadie, et al., 2001; Stenzel, et al., 2020). This immunosuppression predisposes pigeons to secondary infections by pathogens such as *E. coli*, *K. pneumonia*, and rotaviruses (Raue, et al., 2005; Duchatel and Szeleszczuk, 2011). Supporting this hypothesis, rotaviruses were the most abundant eukaryotic RNA viruses (80.43%) detected in the present study. In pigeons, rotaviruses have been associated with enteric diseases, accompanied by symptoms of diarrhoea (Rubbenstroth, et al., 2020; Łukaszuk, et al., 2024). The co-occurrence and dominance of *E. coli*, PiCV, and rotaviruses suggest a potential synergistic pathogenic effect, in which PiCV may facilitate viral and bacterial proliferation, potentially contributing to enteric disease within the pigeon flocks in UTAR Kampar campus. The appearance of collected faecal samples being watery, loose, and bright greenish-yellow in colour (Figure 4.1) could serve as indirect evidence. These features are signs of diarrhoea in pigeons (Paul, et al., 2015; Doneley, 2018; Łukaszuk and Stenzel, 2020). These abnormal droppings were particularly prevalent in samples collected from Blocks C, D, G, N, and P across two collection

batches. However, it is important to note that this association remains hypothetical. Clinical confirmation through pathological and histological evaluations is required to draw a definitive conclusion. Future studies should also investigate the synergistic effects of bacterial and viral co-infections as well as their role in disease progression of avian enteric illness.

CHAPTER 6

CONCLUSION

This study provides a comprehensive overview of the faecal microbiota of rock pigeons (*Columba livia*), in UTAR Kampar campus using shotgun metagenomic sequencing. The bacterial community was phylogenetically more diverse compared to fungal and viral communities. Archaea were not detected in the present study.

The predominant phyla of bacterial community were *Pseudomonadota* (84.44%) and *Bacillota* (15.26%), together accounting for nearly 99.70% of this community. Notably, 38 bacterial species (accounting for 5.11% of the bacterial community) were identified as potential pathogens. Most of them were associated with human infections, mainly affecting the intestinal, urinary, and respiratory tract, which commonly transmitted through the oral-faecal route or via aerosols. Aerosol circulation of airborne pathogens increases the risk of hypersensitivity pneumonitis among nearby individuals. The presence of numerous pathogens should be addressed to prevent the possible zoonotic transmissions, especially in the environments close to dining areas and ventilation systems. Importantly, the detection of 19 AMR genes in rock pigeon droppings suggests their potential role as an environmental reservoir for antimicrobial resistance, which may contribute to the broader issue of emerging multidrug-resistant bacteria. The close proximity of UTAR hospital and UTAR

Kampar campus, which colonised by large pigeon populations, has raises significant public health concerns, particularly for immunocompromised individuals. The possible circulation of AMR genes associated with clinical relevant pathogens highlight the importance of continued surveillance, to mitigate the risk of hospital-associated infections (HAIs).

Fungal detection revealed four genera, which was predominated by *Kazachstania* (94.11%), followed by *Trichosporon* (3.56%), *Debaryomyces* (1.82%), and *Saccharomyces* (0.51%). The low fungal diversity may be attributed to the selection of fresh and high moisture samples, with the removal of uric acids. Two human pathogenic fungi species, *Kazachstania bovina* and *Trichosporon asahii*, contributed 1.69% and 3.56%, respectively to the fungal community. These fungi can cause infections, especially in individuals with underlying health conditions.

Eukaryotic DNA virus community was dominated by pigeon circovirus (73.23%), while eukaryotic RNA virus community was dominated by rotaviruses (80.43%). Out of eleven genera of DNA and RNA viruses detected, six viral genera (comprising nine species) were avian viruses. Seven avian enteric pathogenic viruses, including pigeon parvovirus A, rotaviruses, avian orthoreoviruses and avian astrovirus were detected. Pigeon flocks in UTAR Kampar campus share common environmental resources with other avian species, including both migratory and non-migratory birds. This implies potential virus circulation among the avian populations. Rotavirus A is a zoonotic pathogen. The genotype G18P[17], was proven to exhibit cross-species

pathogenesis in both avian and mammalian models.

The predominance of *Pseudomonadota* together with the co-occurrence of PiCV and rotaviruses, along with the observed diarrheal symptoms, suggests possible health issues among the pigeon flocks. Additionally, a total of twelve enteric pathogenic bacteria and seven enteric pathogenic viruses, were detected. Most of the enteric pathogens can be transmitted to the surrounding populations, including humans through the oral-faecal route. However, it is important to note that this association remains hypothetical. Clinical confirmation through pathological and histological evaluations is required to validate this inference. Future studies should also investigate the synergistic effects of bacterial and viral co-infections as well as their role in disease progression of avian enteric illness.

To mitigate the potential risk of zoonotic transmission, integrated pigeon control strategies are recommended. For example, structural modifications such as ceiling renovation and the installation of exclusion netting can effectively reduce indoor roosting and nesting of pigeon, thus reduce the faecal accumulation. Public education and enforcement of no-feeding policies can also limit the pigeon congregation in human-populated areas.

This study has several limitations. First, the random sample collection without physical assessment on pigeon health issue may introduce selection bias, resulting in a lack of information between microbial diversity distribution in healthy and unhealthy rock pigeons. Second, the exclusion of uric acid, may

lead to the underestimation of fungal diversity. For the virus community, whole genome sequencing for viruses can be conducted in future research to obtain complete genome sequences and further identify novel viruses or to study the evolution of viruses. Nonetheless, shotgun metagenomic sequencing has limitation in distinguish between viable and non-viable microorganisms, thus cannot confirm the phenotypic resistance and virulence. The integration of multiple complementary approaches is essential to achieve an in-depth and insightful research outcomes. For example, cooperate with culturing methos, which the isolated can be further used to verify the activity of pathogens and the multidrug resistance. Long-read sequencing is also recommended to detect the structural variant and to obtain more complete genome assembly.

In a nutshell, this study provides baseline data for microbial risk assessment, and highlight the role of rock pigeon dropping as potential reservoirs for pathogenic and AMR-associated microorganisms. Integrated monitoring, public education, and effective pigeon control are crucial in reducing the risks of zoonotic transmission, particularly in the environments near to dining areas, ventilation systems, and around the healthcare facilities.

REFERENCES

Abadie, J., Nguyen, F., Groizeleau, C., Amenna, N., Fernandez, B., Guereaud, C., Guigand, L., Robart, P., Lefebvre, B. and Wyers, M., 2001. Pigeon circovirus infection: pathological observations and suggested pathogenesis. *Avian Pathology*, [e-journal] 30(2), pp.149-158. https://doi.org/10.1080/03079450124811.

Abulreesh, H.H., 2011. Free living rock pigeon (*Columba livia*) as an environmental
reservoir of Enteric bacterial pathogens resistant to antimicrobial drugs in Saudi Arabia. *Current Research in Bacteriology*, [e-journal] 4(1), pp.28-33. http://dx.doi.org/10.3923/crb.2011.28.33.

Abulreesh, H.H., 2014. Faecal shedding of antibiotic resistant *Escherichia coli* serogroups in pigeons with special reference to *E. coli* O157. *Annual Research and Review in Biology*, [e-journal] 4(13), pp.2184-2191. https://doi.org/10.9734/ARRB/2014/9501.

Abulreesh, H.H., Organji, S.R., Elbanna, K., Osman, G.E., Almalki, M.H., Abdel-Malek, A.Y., Ghyathuddin, A.A. and Ahmad, I., 2019. Diversity, virulence factors, and antifungal
susceptibility patterns of pathogenic and opportunistic yeast species in rock pigeon (*Columba livia*) fecal droppings in Western Saudi Arabia. *Polish Journal of Microbiology*, [e-journal]
68(4), pp.493-504. http://dx.doi.org/10.33073/pjm-2019-049.

Adil, S., 2020. Prevalence and isolation of avian pathogenic *Escherichia coli* from colibacillosis affected broiler chicken in Kashmir Valley. *Life Sciences Leaflets*, [e-journal] 125, pp.06-13.

Ahmed, N.A. and Gulhan, T., 2024. Determination of antibiotic resistance patterns and genotypes of *Escherichia coli* isolated from wild birds. *Microbiome*, [e-journal] 12(1), pp.8. https://doi.org/10.1186/s40168-023-01729-1.

Alav, I., Bavro, V.N. and Blair, J.M., 2021. Interchangeability of periplasmic adaptor proteins *AcrA* and *AcrE* in forming functional efflux pumps with *AcrD* in *Salmonella enterica* serovar Typhimurium. *Journal of Antimicrobial Chemotherapy*, [e-journal] 76(10), pp.2558-2564. https://doi.org/10.1093/jac/dkab237.

Alcalá, L., Alonso, C.A., Simón, C., González-Esteban, C., Orós, J., Rezusta, A., Ortega, C. and Torres, C., 2016. Wild birds, frequent carriers of extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* of CTX-M and SHV-12 types. *Microbial Ecology*, [e-journal] 72, pp.861-869. https://doi.org/10.1093/jac/dkab237.

Alias, N.A. and Hashim, H.S., 2016. House crow presence as unsustainable urban indicator. *International Journal of the Malay World and Civilisation*

(Iman), [e-journal] 4(2016), pp.59-65. http://dx.doi.org/10.17576/IMAN-2016-04SI2-07.

Allan, G.M., Phenix, K.V., Todd, D. and McNulty, M.S., 1994. Some biological and physico-chemical properties of porcine circovirus. *Journal of Veterinary Medicine, Series B*, [e-journal] 41(1-10), pp.17-26. https://doi.org/10.1111/j.1439-0450.1994.tb00201.x.

Al-Tawfiq, J.A. and Ghandour, J., 2007. *Cryptococcus neoformans* abscess and osteomyelitis in an immunocompetent patient with tuberculous lymphadenitis. *Infection*, [e-journal] 35(5), pp.377-382. http://dx.doi.org/10.1007/s15010-007-6109-9.

Andino, A. and Hanning, I., 2015. *Salmonella enterica*: survival, colonization, and virulence differences among serovars. *The Scientific World Journal*, [e-journal] 2015(1), pp.520179. https://doi.org/10.1155/2015/520179.

Annous, B. and Gurtler, J. eds., 2012. *Salmonella: Distribution, Adaptation, Control Measures and Molecular Technologies*. [e-book] BoD: Books on Demand. Available at: Google Books < https://books.google.com.my/books?hl=en&lr=&id=6NqgDwAAQBAJ&oi=fnd&pg=PR11&dq=Salmonella:+Distribution,+Adaptation,+Control+Measures+and+Molecular+Technologies&ots=k0LNVhNqwH&sig=kJreEuz5i73Ll3Hk0l4RiPAlKK4&redir_esc=y#v=onepage&q=Salmonella%3A%20Distribution%2C%20Adaptation%2C%20Control%20Measures%20and%20Molecular%20Technologies&f=false> [Accessed 15 October 2024].

Aranda, P.S., LaJoie, D.M. and Jorcyk, C.L., 2012. Bleach gel: a simple agarose gel for analyzing RNA quality. *Electrophoresis*, [e-journal] 33(2), pp.366-369. https://doi.org/10.1002/elps.201100335.

Armstrong, R.A., 1984. The influence of bird droppings and uric acid on the radial growth of five species of saxicolous lichens. *Environmental and Experimental Botany*, [e-journal] 24(1), pp.95-99. https://doi.org/10.1016/0098-8472(84)90065-0.

Baele, M., Devriese, L.A., Butaye, P. and Haesebrouck, F., 2002. Composition of enterococcal and streptococcal flora from pigeon intestines. *Journal of Applied Microbiology*, [e-journal] *92*(2), pp.348-351. https://doi.org/10.1046/j.1365-2672.2002.01537.x.

Bányai, K., Kemenesi, G., Budinski, I., Földes, F., Zana, B., Marton, S., Varga-Kugler, R., Oldal, M., Kurucz, K. and Jakab, F., 2017. Candidate new rotavirus species in Schreiber's bats, Serbia. *Infection, Genetics and Evolution*, [e-journal] 48, pp.19-26. https://doi.org/10.1016/j.meegid.2016.12.002.

Barka, E.A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.P., Clément, C., Ouhdouch, Y. and van Wezel, G.P., 2016. Taxonomy, physiology, and natural products of Actinobacteria. *Microbiology and Molecular Biology Reviews*, [e-journal] 80(1), pp.1-43.

https://doi.org/10.1128/mmbr.00019-15.

Barroso, K.C., Previato-Mello, M., Batista, B.B., Batista, J.H. and da Silva Neto, J.F., 2018. *EmrR*-dependent upregulation of the efflux pump *EmrCAB* contributes to antibiotic resistance in *Chromobacterium violaceum*. *Frontiers in Microbiology*, [e-journal] 9, pp.2756. https://doi.org/10.3389/fmicb.2018.02756.

BBC, 2019. *Pigeon droppings health risk - should you worry?* [online] Available at: <https://www.bbc.com/news/health-46964702> [Accessed 20 July 2022].

Benson, P.M., Lapins, N.A. and Odom, R.B., 1983. White piedra. *Archives of Dermatology*, [e-journal] 119(7), pp.602-604. https://doi.org/10.1001/archderm.1983.01650310064013

Bhattacharya, I., Yan, S., Yadav, J.S.S., Tyagi, R.D. and Surampalli, R.Y., 2013. *Saccharomyces unisporus*: biotechnological potential and present status. *Comprehensive Reviews in Food Science and Food Safety*, [e-journal] 12(4), pp.353-363. https://doi.org/10.1111/1541-4337.12016.

Binda, C., Lopetuso, L.R., Rizzatti, G., Gibiino, G., Cennamo, V. and Gasbarrini, A., 2018. Actinobacteria: a relevant minority for the maintenance of gut homeostasis. *Digestive and Liver Disease*, [e-journal] 50(5), pp.421-428. https://doi.org/10.1016/j.dld.2018.02.012.

Bishop, R., Davidson, G.P., Holmes, I.H. and Ruck, B., 1973. Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. *The Lancet*, [e-journal] 302(7841), pp.1281-1283. https://doi.org/10.1016/S0140-6736(73)92867-5.

Blakey, J., Crossley, B., Rosenberger, J.K., Rejmanek, D., Markis, M., Bickford, A., Bland, M., Woods, L., Shivaprasad, H.L., Goldsmith, D. and Stoute, S., 2019. Rotavirus A associated with clinical disease and hepatic necrosis in California pigeons (*Columba livia domestica*). *Avian Diseases*, [e-journal] 63(4), pp.651-658. https://doi.org/10.1637/aviandiseases-D-19-00114.

Blanco-Peña, K., Esperón, F., Torres-Mejía, A.M., De la Torre, A., de la Cruz, E. and Jiménez-Soto, M., 2017. Antimicrobial resistance genes in pigeons from public parks in Costa Rica. *Zoonoses and Public Health*, [e-journal] 64(7), pp.23-30. https://doi.org/10.1111/zph.12340.

Blanvillain, C., Saavedra, S., Withers, T., Votýpka, J., Laroucau, K., Lowenski, S. and Modrý, D., 2021. Screening of diseases in wild exotic birds on Tahiti Island–implications for French Polynesian conservation. *Pacific Conservation Biology*. [e-journal] 27(3), pp.284-295. https://doi.org/10.1071/pc20049.

Blitvich, B.J., 2008. Transmission dynamics and changing epidemiology of West Nile virus. *Animal Health Research Reviews*, [e-journal] 9(1), pp.71-86. https://doi.org/10.1017/S1466252307001430.

Bonnedahl, J. and Järhult, J.D., 2014. Antibiotic resistance in wild birds. *Upsala journal of medical sciences*, [e-journal] 119(2), pp.113-116. https://doi.org/10.3109/03009734.2014.905663.

Borer, A., Saidel-Odes, L., Riesenberg, K., Eskira, S., Peled, N., Nativ, R., Schlaeffer, F. and Sherf, M., 2009. Attributable mortality rate for carbapenem-resistant *Klebsiella pneumoniae* bacteremia. *Infection Control and Hospital Epidemiology*, [e-journal] 30(10), pp.972-976. https://doi.org/10.1086/605922.

Bose, T., Borrow, R. and Arkwright, P.D., 2024. Impact of rotavirus vaccination on diarrheal disease burden of children in South America. *Expert Review of Vaccines*, [e-journal] 23(1), pp.584-596. https://doi.org/10.1080/14760584.2024.2360212.

Bourke, S.J., Dalphin, J.C., Boyd, G., McSharry, C., Baldwin, C.I. and Calvert, J.E., 2001. Hypersensitivity pneumonitis: current concepts. *European Respiratory Journal*, 18(32 suppl), pp.81S-92S.

Breitbart, M., Delwart, E., Rosario, K., Segalés, J., Varsani, A. and ICTV Report Consortium, 2017. ICTV virus taxonomy profile: *Circoviridae*. *Journal of General Virology*, 98(8), pp.1997-1998.

Brown, J.D., Stallknecht, D.E., Berghaus, R.D. and Swayne, D.E., 2009. Infectious and lethal doses of H5N1 highly pathogenic avian influenza virus for house sparrows (*Passer domesticus*) and rock pigeons (*Columbia livia*). *Journal of Veterinary Diagnostic Investigation*, [e-journal] 21(4), pp.437-445. https://doi.org/10.1177/10406387090210040.

Bruun, J. E., n.d. *Feral pigeon*. [online] Available at: <https://luontoportti.com/en/t/686/feral-pigeon> [Accessed 19 July 2022].

Bupasha, Z.B., Begum, R., Karmakar, S., Akter, R. and Ahad, A., 2020. Multidrug-resistant *Salmonella* spp. isolated from apparently healthy pigeons in a live bird market in Chattogram, Bangladesh. *World's Veterinary Journal*, [e-journal] 10(4), pp.508-513. https://dx.doi.org/10.54203/scil.2020.wvj61.

Burt, S.A., Röring, R.E. and Heijne, M., 2018. *Chlamydia psittaci* and *C. avium* in feral pigeon (*Columba livia domestica*) droppings in two cities in the Netherlands. *Veterinary Quarterly*, [e-journal] 38(1), pp.63-66. https://doi.org/10.1080/01652176.2018.1482028.

Busi, C., Martella, V., Papetti, A., Sabelli, C., Lelli, D., Alborali, G.L., Gibelli, L., Gelmetti, D., Lavazza, A., Cordioli, P. and Boniotti, M.B., 2017. Group A rotavirus associated with encephalitis in red fox. *Emerging Infectious Diseases*, [e-journal] 23(9), pp.1535-1538. https://doi.org/10.3201/eid2309.170158.

Butz, A.M., Fosarelli, P., Dick, J., Cusack, T. and Yolken, R., 1993. Prevalence of rotavirus on high-risk fomites in day-care facilities. *Pediatrics*, [e-journal] 92(2), pp.202-205. https://doi.org/10.1542/peds.92.2.202.

Camacho-Muñoz, D., Martín, J., Santos, J.L., Aparicio, I. and Alonso, E., 2010. Occurrence, temporal evolution and risk assessment of pharmaceutically active compounds in Doñana Park (Spain). *Journal of Hazardous Materials*, [e-journal] 183(1-3), pp.602-608. https://doi.org/10.1016/j.jhazmat.2010.07.067.

Cao, J., Hu, Y., Liu, F., Wang, Y., Bi, Y., Lv, N., Li, J., Zhu, B. and Gao, G.F., 2020. Metagenomic analysis reveals the microbiome and resistome in migratory birds. *Microbiome,* [e-journal] 8(26), pp.1-18. https://doi.org/10.1186/s40168-019-0781-8.

Capua, I. and Alexander, D.J., 2004. Human health implications of avian influenza viruses and paramyxoviruses. *European Journal of Clinical Microbiology and Infectious Diseases*, [e-journal] 23(1), pp.1-6. https://doi.org/10.1007/s10096-003-1059-3.

Carroll, D., Wang, J., Fanning, S. and McMahon, B.J., 2015. Antimicrobial resistance in wildlife: implications for public health. *Zoonoses and Public Health*, [e-journal] 62(7), pp.534-542. https://doi.org/10.1111/zph.12182.

Castano G, Yarrarapu SNS, Mada PK., 2023. *Trichosporonosis*. [StatPearls] NCBI. Available at: Ncbi.nlm.nih.gov <https://www.ncbi.nlm.nih.gov/books/NBK482477/> [Accessed 10 October 2024].

CDC, 2020. *Histoplasmosis*. [online] Available at: <https://www.cdc.gov/fungal/diseases/histoplasmosis/index.html#:~:text=Histoplasmosis%20is%20an%20infection%20caused,of%20bird%20or%20bat%20droppings.> [Accessed 25 February 2024].

Chan, Y., 2020. *Get rid of these disease-carrying 'flying rats'*. [online] Available at: < https://www.thestar.com.my/opinion/letters/2020/07/24/get-rid-of-these-disease-carrying-flying-rats> [Accessed 7 August 2022].

Chen, W.T., Teng, C.A., Shih, C.H., Huang, W.H., Jiang, Y.F., Chang, H.W., Jeng, C.R., Lai, Y.H., Guo, J.C., Wang, P.J. and Cheng, C.H., 2021. Investigation of lethal concurrent outbreak of chlamydiosis and pigeon circovirus in a zoo. *Animals*, [e-journal] 11(6), pp.1654. https://doi.org/10.3390/ani11061654.

Cheng, Y.J., Lin, K.Y., Chen, C.C., Huang, Y.L., Liu, C.E. and Li, S.Y., 2013. Zoonotic atypical pneumonia due to *Chlamydophila psittaci*: first reported psittacosis case in Taiwan. *Journal of the Formosan Medical Association*, [e-journal] 112(7), pp.430-433. https://doi.org/10.1016/j.jfma.2012.08.017.

Chistoserdova, L., 2009. Functional metagenomics: recent advances and future challenges. *Biotechnology and Genetic Engineering Reviews*, [e-journal] 26(1), pp.335-352. https://doi.org/10.5661/bger-26-335.

Chitty, J., 2018. Pigeons (*Columba livia*). *Companion Animal Care and Welfare: The UFAW Companion Animal Handbook,* [e-journal] pp.355-370. https://doi.org/10.1002/9781119333708.ch17.

Chua, K.B., 2003. A novel approach for collecting samples from fruit bats for isolation of infectious agents. *Microbes and Infection*, [e-journal] 5(6), pp.487-490. https://doi.org/10.1016/S1286-4579(03)00067-4.

Chua, K.B., Crameri, G., Hyatt, A., Yu, M., Tompang, M.R., Rosli, J., McEachern, J., Crameri, S., Kumarasamy, V., Eaton, B.T. and Wang, L.F., 2007. A previously unknown reovirus of bat origin is associated with an acute respiratory disease in humans. *Proceedings of the National Academy of Sciences*, [e-journal] 104(27), pp.11424-11429. https://doi.org/10.1073/pnas.0701372104.

Chua, K.B., Voon, K., Crameri, G., Tan, H.S., Rosli, J., McEachern, J.A., Suluraju, S., Yu, M. and Wang, L.F., 2008. Identification and characterization of a new orthoreovirus from patients with acute respiratory infections. *PloS One*, [e-journal] 3(11), pp.3803. https://doi.org/10.1371/journal.pone.0003803.

Cleveland Clinic medical, 2022. *Salmonella*. [online] Available at: <https://my.clevelandclinic.org/health/diseases/15697-salmonella> [Accessed 15 November 2022].

Coburn, B., Grassl, G.A. and Finlay, B.B., 2007. *Salmonella*, the host and disease: a brief review. *Immunology and Cell Biology*, [e-journal] 85(2), pp.112-118. https://doi.org/10.1038/sj.icb.7100007.

Cogliati, M., 2013. Global molecular epidemiology of *Cryptococcus neoformans* and *Cryptococcus gattii*: an atlas of the molecular types. *Scientifica*, [e-journal] 2013(1), pp.1-23. https://doi.org/10.1155/2013/675213.

Coletti, M., Franciosini, M.P., Asdrubali, G. and Passamonti, F., 2000. Atrophy of the primary lymphoid organs of meat pigeons in Italy associated with circoviruslike particles in the bursa of Fabricius. *Avian Diseases*, [e-journal] 44(2), pp.454-459. https://doi.org/10.2307/1592562.

Colombo, A.L., Padovan, A.C.B. and Chaves, G.M., 2011. Current knowledge of *Trichosporon* spp. and Trichosporonosis. *Clinical Microbiology Reviews*, [e-journal] 24(4), pp.682-700. https://doi.org/10.1128/cmr.00003-11.

Colpitts, T.M., Conway, M.J., Montgomery, R.R. and Fikrig, E., 2012. West Nile Virus: biology, transmission, and human infection. *Clinical Microbiology Reviews*, [e-journal] 25(4), pp.635-648. https://doi.org/10.1128/cmr.00045-12.

Colvin, L.B., Creger, C.R., Couch, J.R., Ferguson, T.M. and Ansari, M.N.A., 1966. A simplified method for separation of urine and feces in the immature fowl. *Proceedings of the Society for Experimental Biology and Medicine*, [e-journal] 123(2), pp.415-417. https://doi.org/10.3181/00379727-123-31501.

Cooper, J.E., 1996. Health studies on the Indian house crow (*Corvus splendens*). *Avian Pathology*, [e-journal] 25(2), pp.381-386. https://doi.org/10.1080/03079459608419148.

Cordero, J., Alonso-Calleja, C., García-Fernández, C. and Capita, R., 2019.

Microbial load and antibiotic resistance patterns of *Escherichia coli* and *Enterococcus faecalis* isolates from the meat of wild and domestic pigeons. *Foods*, [e-journal] 8(11), pp.536. https://doi.org/10.3390/foods8110536.

Costa, A.K., Sidrim, J.J., Cordeiro, R.A., Brilhante, R.S., Monteiro, A.J. and Rocha, M.F., 2010. Urban pigeons (*Columba livia*) as a potential source of pathogenic yeasts: a focus on antifungal susceptibility of *Cryptococcus* strains in Northeast Brazil. *Mycopathologia*, [e-journal] 169(3), pp.207-213. https://doi.org/10.1007/s11046-009-9245-1.

Cotmore, S.F., Agbandje-McKenna, M., Canuti, M., Chiorini, J.A., Eis-Hubinger, A.M., Hughes, J., Mietzsch, M., Modha, S., Ogliastro, M., Pénzes, J.J. and Pintel, D.J., 2019. ICTV virus taxonomy profile: Parvoviridae. *Journal of General Virology*, 100(3), pp.367-368.

Cramer, C., Schlünssen, V., Bendstrup, E., Stokholm, Z.A., Vestergaard, J.M., Frydenberg, M. and Kolstad, H.A., 2016. Risk of hypersensitivity pneumonitis and interstitial lung diseases among pigeon breeders. *European Respiratory Journal*, [e-journal] 48(3), pp.818-825. https://doi.org/10.1183/13993003.00376-2016.

Critchley, K., 2021. *Why are bird droppings mostly white, considering the things birds eat?*. [online] Available at: <https://www.newscientist.com/lastword/mg25133461-700-why-are-birddroppings-mostly-white-considering-the-things-birds-eat/> [Accessed 17 November 2022].

Croxen, M.A., Law, R.J., Scholz, R., Keeney, K.M., Wlodarska, M. and Finlay, B.B., 2013. Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clinical Microbiology Reviews*, [e-journal] 26(4), pp.822-880. https://doi.org/10.1128/CMR.00022-13.

CZ ID, n.d. *CZ ID Pipeline Overviews.* [online] Available at: <https://chanzuckerberg.zendesk.com/hc/en-us/articles/13756558532884-CZ-ID-Pipeline-Overviews> [Accessed 20 May 2023].

da Silva, V.L., Caçador, N.C., da Silva, C.D.S.F., Fontes, C.O., Garcia, G.D., Nicoli, J.R. and Diniz, C.G., 2012. Occurrence of multidrug-resistant and toxic-metal tolerant *enterococci* in fresh feces from urban pigeons in Brazil. *Microbes and Environments*, [e-journal] 27(2), pp.179-185. https://doi.org/10.1264/jsme2.ME11296.

Dai, N., Li, Q., Geng, J., Guo, W. and Yan, W., 2022. Severe pneumonia caused by *Chlamydia psittaci*: report of two cases and literature review. *The Journal of Infection in Developing Countries*, [e-journal] 16(06), pp.1101-1112. https://doi.org/10.3855/jidc.16166.

De Briyne, N., Atkinson, J., Borriello, S.P. and Pokludová, L., 2014. Antibiotics used most commonly to treat animals in Europe. *Veterinary Record*, [e-journal] 175(13), pp.325-325. https://doi.org/10.1136/vr.102462.

De Leener, K., Rahman, M., Matthijnssens, J., Van Hoovels, L., Goegebuer, T., Van der Donck, I. and Van Ranst, M., 2004. Human infection with a P [14], G3 lapine rotavirus. *Virology*, [e-journal] 325(1), pp.11-17. https://doi.org/10.1016/j.virol.2004.04.020.

De Oliveira, M.C., Camargo, B.Q., Cunha, M.P., Saidenberg, A.B., Teixeira, R.H., Matajira, C.E., Moreno, L.Z., Gomes, V.T., Christ, A.P., Barbosa, M.R. and Sato, M.I., 2018. Free-ranging synanthropic birds (*Ardea alba* and *Columba livia domestica*) as carriers of *Salmonella* spp. and diarrheagenic *Escherichia coli* in the vicinity of an urban zoo. *Vector-Borne and Zoonotic Diseases*, [e-journal] 18(1), pp.65-69. https://doi.org/10.1089/vbz.2017.2174.

De Vuyst, L. and Neysens, P., 2005. The sourdough microflora: biodiversity and metabolic interactions. *Trends in Food Science and Technology*, [e-journal] 16(1-3), pp.43-56. https://doi.org/10.1016/j.tifs.2004.02.012.

Dennehy, P.H., Nelson, S.M., Crowley, B.A. and Saracen, C.L., 1998. Detection of rotavirus RNA in hospital air samples by polymerase chain reaction (PCR)•828. *Pediatric Research*, [e-journal] 43(4), pp.143-143. https://doi.org/10.1203/00006450-199804001-00849.

Desselberger, U., 2014. Rotaviruses. *Virus Research*, [e-journal] 190, pp.75-96. https://doi.org/10.1016/j.virusres.2014.06.016.

Dey, R.K., Khatun, M.M., Islam, M.A. and Hosain, M.S., 2013. Prevalence of multidrug resistant *Escherichia coli* in pigeon in Mymensingh, Bangladesh. *Microbes and Health*, [e-journal] 2(1), pp.5-7. https://doi.org/10.3329/mh.v2i1.17254.

Dhama, K., Saminathan, M., Karthik, K., Tiwari, R., Shabbir, M.Z., Kumar, N., Malik, Y.S. and Singh, R.K., 2015. Avian rotavirus enteritis–an updated review. *Veterinary Quarterly*, [e-journal] 35(3), pp.142-158. http://www.tandfonline.com/doi/full/10.1080/01652176.2015.1046014.

Dietz, M.W., Matson, K.D., Versteegh, M.A., Van Der Velde, M., Parmentier, H.K., Arts, J.A., Salles, J.F. and Tieleman, B.I., 2022. Gut microbiota of homing pigeons shows summer–winter variation under constant diet indicating a substantial effect of temperature. *Animal Microbiome*, [e-journal] 4(1), pp.64. https://doi.org/10.1186/s42523-022-00216-6.

Ding, J., Liao, N., Zheng, Y., Yang, L., Zhou, H., Xu, K., Han, C., Luo, H., Qin, C., Tang, C. and Wei, L., 2020. The composition and function of pigeon milk microbiota transmitted from parent pigeons to squabs. *Frontiers in Microbiology*, [e-journal] 11, pp.1789. https://doi.org/10.3389/fmicb.2020.01789.

Dolka, B., Czopowicz, M., Chrobak-Chmiel, D., Ledwoń, A. and Szeleszczuk, P., 2020. Prevalence, antibiotic susceptibility and virulence factors of *Enterococcus* species in racing pigeons (*Columba livia f. domestica*). *BMC Veterinary Research*, [e-journal] 16, pp.1-14. https://doi.org/10.1186/s12917-019-2200-6.

Doneley, B., 2018. *Avian medicine and surgery in practice: companion and aviary birds*. CRC press.

Dong, H.V., Tran, G.T.H., Nguyen, H.T.T., Nguyen, T.M., Trinh, D.Q., Le, V.P., Choowongkomon, K. and Rattanasrisomporn, J., 2022. Epidemiological analysis and genetic characterization of parvovirus in ducks in Northern Vietnam reveal evidence of recombination. *Animals*, [e-journal] 12(20), pp.2846. https://doi.org/10.3390/ani12202846.

Donlan, R.M., 2001. Biofilm formation: a clinically relevant microbiological process. *Clinical Infectious Diseases*, [e-journal] 33(8), pp.1387-1392. https://doi.org/10.1086/322972.

Dovč, A., Jereb, G., Krapež, U., Gregurić-Gračner, G., Pintarič, Š., Slavec, B., Knific, R.L., Kastelic, M., Kvapil, P., Mićunović, J. and Vadnjal, S., 2016. Occurrence of bacterial and viral pathogens in common and noninvasive diagnostic sampling from parrots and racing pigeons in Slovenia. *Avian Diseases*, [e-journal] 60(2), pp.487-492. https://doi.org/10.1637/11373-011116-Reg.

Drlica, K. and Zhao, X., 1997. DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiology and Molecular Biology Reviews*, [e-journal] 61(3), pp.377-392. https://doi.org/10.1128/mmbr.61.3.377-392.1997.

Duchatel, J.P. and Szeleszczuk, P., 2011. Young pigeon disease syndrome. *Medycyna Weterynaryjna*, 67(5), pp.291-294.

Duchatel, J.P., Todd, D., Smyth, J.A., Bustin, J.C. and Vindevogel, H., 2006. Observations on detection, excretion and transmission of pigeon circovirus in adult, young and embryonic pigeons. *Avian Pathology*, [e-journal] 35(1), pp.30-34. https://doi.org/10.1080/03079450500465692.

Dutta, P., Borah, M., Sarmah, R. and Gangil, R., 2013. Isolation of *Salmonella* Typhimurium from pigeons (*Columba livia*) in Greater Guwahati, its histopathological impact and antibiogram. *Comparative Clinical Pathology*, [e-journal] 22(1), pp.147-150. https://doi.org/10.1007/s00580-012-1614-3.

Eguchi, Y., Ishii, E., Hata, K. and Utsumi, R., 2011. Regulation of acid resistance by connectors of two-component signal transduction systems in *Escherichia coli*. *Journal of Bacteriology*, [e-journal] 193(5), pp.1222-1228. https://doi.org/10.1128/jb.01124-10.

Eguchi, Y., Oshima, T., Mori, H., Aono, R., Yamamoto, K., Ishihama, A. and Utsumi, R., 2003. Transcriptional regulation of drug efflux genes by EvgAS, a two-component system in *Escherichia coli*. *Microbiology*, [e-journal] 149(10), pp.2819-2828. https://doi.org/10.1099/mic.0.26460-0.

Ellis, T.M., Barry Bousfield, R., Bissett, L.A., Dyrting, K.C., Luk, G.S., Tsim, S.T., Sturm-Ramirez, K., Webster, R.G., Guan, Y. and Peiris, J.M., 2004. Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. *Avian Pathology*, [e-

journal] 33(5), pp.492-505. https://doi.org/10.1080/03079450400003601.

Enne, V.I., Delsol, A.A., Roe, J.M. and Bennett, P.M., 2006. Evidence of antibiotic resistance gene silencing in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy*, [e-journal] 50(9), pp.3003-3010. https://doi.org/10.1128/aac.00137-06.

Environmental and Disaster Restoration (ECOS), n.d. Pigeon Droppings Cleanup. [Online] Available at: <https://ecosenvironmental.com/pigeon-droppings/> [Accessed 15 Norvember 2022].

Esher, S.K., Zaragoza, O. and Alspaugh, J.A., 2018. Cryptococcal pathogenic mechanisms: a dangerous trip from the environment to the brain. *Memorias do Instituto Oswaldo Cruz,* [e-journal] 113(7), pp.180057. https://doi.org/10.1590/0074-02760180057.

Esposito, E., Scarpellini, R., Celli, G., Marliani, G., Zaghini, A., Mondo, E., Rossi, G. and Piva, S., 2024. Wild birds as potential bioindicators of environmental antimicrobial resistance: A preliminary investigation. *Research in Veterinary Science*, [e-journal] 180, pp.105424. https://doi.org/10.1016/j.rvsc.2024.105424.

European Centre for Disease Prevention and Control, 2018. Antimicrobial Consumption—Annual Epidemiological Report for 2017.

Fàbrega, A. and Vila, J., 2013. *Salmonella enterica* serovar Typhimurium skills to succeed in the host: virulence and regulation. *Clinical Microbiology Reviews*, [e-journal] 26(2), pp.308-341. https://doi.org/10.1128/cmr.00066-12.

Facciolà, A., Ceccarelli, M., Venanzi Rullo, E., d'Aleo, F., Paolucci, I.A., D'Andrea, F., Momdello, P., Lo Presti, M.R., Marabto, D. and Pellicanò, G.F., 2019. An evaluation of the burden of invasive *Enterococcal* infection in a large university hospital. [e-journal] *Infectious Diseases and Tropical Medicine*, 5(523), pp.1-6. https://doi.org/10.32113/idtm_20195_523.

Fahey, J.E. and Crawley, J.F., 1954. Studies on chronic respiratory disease of chickens II. isolation of a virus. *Canadian Journal of Comparative Medicine and Veterinary Science*, 18(1), pp.13.

Fan, W., Zhu, Y., Hou, H., Yao, J., Zhu, L., Liu, H. and Yan, H., 2024. Treatment and prevention of pigeon diarrhea through the application of *Lactobacillus* SNK-6. *Poultry Science*, [e-journal] 103(4), pp.103476. https://doi.org/10.1016/j.psj.2024.103476.

Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U. and Ball, L.A. eds., 2005. *Virus taxonomy: VIIIth report of the International Committee on Taxonomy of Viruses*. [e-book] Academic Press. Available at: Google Books < https://books.google.com.my/books?hl=en&lr=&id=9Wy7Jgy5RWYC&oi=fnd&pg=PP1&dq=Virus+taxonomy:+VIIIth+report+of+the+International+Committee+on+Taxonomy+of+Viruses.+&ots=FTAvkiu_3d&sig=_SJ_13lf6djmnGjV7tuQGxvojhE&redir_esc=y#v=onepage&q=Virus%20taxonomy%3A%20

VIIIth%20report%20of%20the%20International%20Committee%20on%20Taxonomy%20of%20Viruses.&f=false> [Accessed 15 May 2024].

Feye, K.M., Baxter, M.F.A., Tellez-Isaias, G., Kogut, M.H. and Ricke, S.C., 2020. Influential factors on the composition of the conventionally raised broiler gastrointestinal microbiomes. *Poultry Science*, [e-journal] 99(2), pp.653-659. https://doi.org/10.1016/j.psj.2019.12.013.

Fraser, D.L., Aguilar, G., Nagle, W., Galbraith, M. and Ryall, C., 2015. The house crow (*Corvus splendens*): a threat to New Zealand?. *ISPRS International Journal of Geo-Information*, [e-journal] 4(2), pp.725-740. https://doi.org/10.3390/ijgi4020725.

Fukui, S., Kawamura, W., Uehara, Y. and Naito, T., 2021. A patient with psittacosis from a pigeon: A reminder of the importance of detailed interviews and relative bradycardia. *IDCases*, [e-journal] 25, pp.01164. https://doi.org/10.1016/j.idcr.2021.e01164.

Gal-Mor, O., Boyle, E.C. and Grassl, G.A., 2014. Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella enterica* serovars differ. *Frontiers in Microbiology*, [e-journal] *5*, pp.391. https://doi.org/10.3389/fmicb.2014.00391.

García-Rowe, J. and Sáiz-Jiménez, C., 1991. Lichens and bryophytes as agents of deterioration of building materials in Spanish cathedrals. *International Biodeterioration*, [e-journal] 28(1-4), pp.151-163. https://doi.org/10.1016/0265-3036(91)90040-X.

Gard, G.P. and Marshall, I.D., 1973. Nelson Bay virus: a novel reovirus. *Archiv für die gesamte Virusforschung*, [e-journal] 43, pp.34-42. https://doi.org/10.1007/BF01249346.

Gatti, M., Silverio, A.D., Cespa, M. and Mosca, M., 1997. Primary unusual cutaneous cryptococcosis in an HIV former drug-abuser patient. *Mycoses*, [e-journal] 40(3-4), pp.101-102. https://doi.org/10.1111/j.1439-0507.1997.tb00195.x.

Gentsch, J.R., Laird, A.R., Bielfelt, B., Griffin, D.D., Bányai, K., Ramachandran, M., Jain, V., Cunliffe, N.A., Nakagomi, O., Kirkwood, C.D. and Fischer, T.K., 2005. Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. *Journal of Infectious Diseases*, [e-journal] 192(Supplement_1), pp.S146-S159. https://doi.org/10.1086/431499.

Gharajalar, S.N. and Shahbazi, P., 2020. Distribution of oxytetracycline resistance genes in *E. coli* isolated from pigeon faecal samples. *Journal of Exotic Pet Medicine*, [e-journal] 33, pp.14-17. https://doi.org/10.1053/j.jepm.2019.12.005.

Giunchi, D., Albores-Barajas, Y.V., Baldaccini, N.E., Vanni, L. and Soldatini, C., 2012. Feral pigeons: problems, dynamics and control methods. *Integrated*

Pest Management and Pest Control. Current and future tactics, London, InTechOpen, pp.215-240.

Goebel, S.J., Taylor, J., Barr, B.C., Kiehn, T.E., Castro-Malaspina, H.R., Hedvat, C.V., RushWilson, K.A., Kelly, C.D., Davis, S.W., Samsonoff, W.A. and Hurst, K.R., 2007. Isolation of avian paramyxovirus 1 from a patient with a lethal case of pneumonia. *Journal of Virology*, [e-journal] 81(122), pp.12709-12714. https://doi.org/10.1128/JVI.01406-07.

Goktas, H., Dikmen, H., Demirbas, F., Sagdic, O. and Dertli, E., 2021. Characterisation of probiotic properties of yeast strains isolated from kefir samples. *International Journal of Dairy Technology*, [e-journal] 74(4), pp.715-722. https://doi.org/10.1111/1471-0307.12802.

Gonçalves, A., Igrejas, G., Radhouani, H., Santos, T., Monteiro, R., Pacheco, R., Alcaide, E., Zorrilla, I., Serra, R., Torres, C. and Poeta, P., 2013. Detection of antibiotic resistant *enterococci* and *Escherichia coli* in free range Iberian Lynx (*Lynx pardinus*). *Science of the Total Environment*, [e-journal] 456, pp.115-119. https://doi.org/10.1016/j.scitotenv.2013.03.073.

Grond, K., Perreau, J.M., Loo, W.T., Spring, A.J., Cavanaugh, C.M. and Hird, S.M., 2019. Longitudinal microbiome profiling reveals impermanence of probiotic bacteria in domestic pigeons. *PLoS One*, [e-journal] 14(6), pp.0217804. https://doi.org/10.1371/journal.pone.0220347.

Grond, K., Sandercock, B.K., Jumpponen, A. and Zeglin, L.H., 2018. The avian gut microbiota: community, physiology and function in wild birds. *Journal of Avian Biology*, [e-journal] 49(11), pp.01788. https://doi.org/10.1111/jav.01788.

Gronesova, P., Mizakova, A. and Betakova, T., 2009. Determination of hemagglutinin and neuraminidase subtypes of avian influenza A viruses in urban pigeons by a new nested RT-PCR. *Acta Virologica*, [e-journal] 53(3), pp.213. https://doi.org/10.4149/av_2009_03_213.

Guerrero-Ramos, E., Cordero, J., Molina-González, D., Poeta, P., Igrejas, G., Alonso-Calleja, C. and Capita, R., 2016. Antimicrobial resistance and virulence genes in *enterococci* from wild game meat in Spain. *Food Microbiology*, [e-journal] 53, pp.156-164. https://doi.org/10.1016/j.fm.2015.09.007.

Guglielmi, K.M., Johnson, E.M., Stehle, T. and Dermody, T.S., 2006. Attachment and cell entry of mammalian orthoreovirus. *Reoviruses: Entry, Assembly and Morphogenesis*, [e-journal] 309, pp.1-38. https://doi.org/10.1007/3-540-30773-7_1.

Haag-Wackernagel, D. and Moch, H., 2004. Health hazards posed by feral pigeons. *Journal of Infection*, [e-journal] 48(4), pp.307-313. https://doi.org/10.1016/j.jinf.2003.11.001.

Hale TL, Keusch GT.,1996. *Shigella*. [Medical Microbiology] Galveston (TX): University of Texas Medical Branch at Galveston. Available at: Ncbi.nlm.nih.gov <https://www.ncbi.nlm.nih.gov/books/NBK8038/>

[Accessed 5 May 2024].

Harkinezhad, T., Verminnen, K., De Buyzere, M., Rietzschel, E., Bekaert, S. and Vanrompay, D., 2009. Prevalence of *Chlamydophila psittaci* infections in a human population in contact with domestic and companion birds. *Journal of Medical Microbiology*, [e-journal] 58(9), pp.1207-1212. https://doi.org/10.1099/jmm.0.011379-0.

Harris, S., n.d. *Feral pigeon: flying rat or urban hero?*. [online] Available at: <https://www.discoverwildlife.com/animal-facts/birds/feral-pigeon-flying-rat-or-urban-hero/> [Accessed 7 August 2022].

Harzer, M., Heenemann, K., Sieg, M., Vahlenkamp, T., Freick, M. and Rückner, A., 2021. Prevalence of pigeon rotavirus infections: animal exhibitions as a risk factor for pigeon flocks. *Archives of Virology*, [e-journal] 166, pp.65-72. https://doi.org/10.1007/s00705-020-04834-w.

He, C., Feng, J., Su, J., Zhang, T. and Yu, L., 2022. Application of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for the rapid identification of yeast species from polar regions. *Frontiers in Microbiology*, [e-journal] 13, pp.832893. https://doi.org/10.3389/fmicb.2022.832893.

Heddema, E.R., Ter Sluis, S., Buys, J.A., Vandenbroucke-Grauls, C.M., van Wijnen, J.H. and Visser, C.E., 2006. Prevalence of *Chlamydophila psittaci* in fecal droppings from feral pigeons in Amsterdam, The Netherlands. *Applied and Environmental Microbiology*, [e-journal] 72(6), pp.4423-4425. https://doi.org/10.1128/AEM.02662-05.

Henry, K. and Crossley, K., 1986. Wild-pigeon-related psittacosis in a family. *Chest*, [e-journal] 90(5), pp.708-710. https://doi.org/10.1378/chest.90.5.708.

Hernandez, J., Johansson, A., Stedt, J., Bengtsson, S., Porczak, A., Granholm, S., Gonzalez-Acuna, D., Olsen, B., Bonnedahl, J. and Drobni, M., 2013. Characterization and comparison of extended-spectrum β-lactamase (ESBL) resistance genotypes and population structure of *Escherichia coli* isolated from Franklin's gulls (*Leucophaeus pipixcan*) and humans in Chile. *PloS one*, [e-journal] 8(9), pp.76150. https://doi.org/10.1371/journal.pone.0076150.

Hirsch, E.B. and Tam, V.H., 2010. Detection and treatment options for *Klebsiella pneumoniae* carbapenemases (KPCs): an emerging cause of multidrug-resistant infection. *Journal of Antimicrobial Chemotherapy*, [e-journal] 65(6), pp.1119-1125. https://doi.org/10.1093/jac/dkq108.

Hommais, F., Krin, E., Laurent-Winter, C., Soutourina, O., Malpertuy, A., Le Caer, J.P., Danchin, A. and Bertin, P., 2001. Large-scale monitoring of pleiotropic regulation of gene expression by the prokaryotic nucleoid-associated protein, H-NS. *Molecular Microbiology*, [e-journal] 40(1), pp.20-36. https://doi.org/10.1046/j.1365-2958.2001.02358.x.

Hosseinian, S.A., 2022. Zoonotic diseases associated with pet birds. *Journal of Zoonotic Diseases*, [e-journal] 6(3), pp.91-112. https://doi.org/10.22034/jzd.2022.15461.

Hunnam, J.C., Sloan, S., McCowan, C.I., Glass, E. and Walker, C., 2019. The racing pigeon (*Columba livia domestica*) industry in Victoria, Australia, and epidemiology of a novel Group A rotavirus outbreak. *Transboundary and Emerging Diseases*, [e-journal] 66(5), pp.2058-2066. https://doi.org/10.1111/tbed.13254.

Inada, S., Okajima, T., Utsumi, R. and Eguchi, Y., 2021. Acid-sensing histidine kinase with a redox switch. *Frontiers in Microbiology*, [e-journal] 12, pp.652546. https://doi.org/10.3389/fmicb.2021.652546.

Ingerson-Mahar, M. and Reid, A., 2011. *FAQ: E. coli: good, bad, and deadly*. [online] Available at: <https://asm.org/Reports/FAQ-E-Coli-Good,-Bad,-and-Deadly> [Accessed 26 March 2024].

International Committee on Taxonomy of Viruses (ICTV), 2024. *Genus: Orthoreovirus*. [online] Available at: <https://ictv.global/report/chapter/spinareoviridae/spinareoviridae/orthoreovirus> [Accessed 5 October 2024].

Irga, P.J., Armstrong, B., King, W.L., Burchett, M. and Torpy, F.R., 2016. Correspondence between urban bird roosts and the presence of aerosolised fungal pathogens. *Mycopathologia*, [e-journal] 181, pp.689-699. https://doi.org/10.1007/s11046-016-0013-8.

Ito, H., Sugiyama, M., Masubuchi, K., Mori, Y. and Minamoto, N., 2001. Complete nucleotide sequence of a group A avian rotavirus genome and a comparison with its counterparts of mammalian rotaviruses. *Virus Research*, [e-journal] 75(2), pp.123-138. https://doi.org/10.1016/S0168-1702(01)00234-9.

Jager, M.C., Tomlinson, J.E., Lopez-Astacio, R.A., Parrish, C.R. and Van de Walle, G.R., 2021. Small but mighty: old and new parvoviruses of veterinary significance. *Virology Journal*, [e-journal] 18, pp.1-29. https://doi.org/10.1186/s12985-021-01677-y.

James, S.A., Carvajal Barriga, E.J., Portero Barahona, P., Nueno-Palop, C., Cross, K., Bond, C.J. and Roberts, I.N., 2015. *Kazachstania yasuniensis* sp. nov., an ascomycetous yeast species found in mainland Ecuador and on the Galápagos. *International Journal of Systematic and Evolutionary Microbiology*, [e-journal] 65(4), pp.1304-1309. https://doi.org/10.1099/ijs.0.000102.

Japa, I., Ahmed, D., Fernandez, A., Alvarez, A., Japa, S.S., Cordero, R., Acosta, F., Steenhoff, A.P. and Lowenthal, E.D., 2024. Etiologies of Mild and Moderate Diarrheal Illness among Children in Consuelo, Dominican Republic. *The American Journal of Tropical Medicine and Hygiene*, [e-journal] 110(2), pp.339-345. https://doi.org/10.4269/ajtmh.23-0299.

Jarma, D., Sánchez, M.I., Green, A.J., Peralta-Sánchez, J.M., Hortas, F., Sánchez-Melsió, A. and Borrego, C.M., 2021. Faecal microbiota and antibiotic resistance genes in migratory waterbirds with contrasting habitat use. *Science of the Total Environment*, [e-journal] 783, pp.146872. https://doi.org/10.1016/j.scitotenv.2021.146872.

Jeyarajasingam, A. (2012). *A field guide to the birds of Peninsular Malaysia and Singapore*. [e-book] Oxford University Press. Available at: Google Books <https://books.google.com.my/books?id=UIh8RwJpYYkC&printsec=copyright&redir_esc=y#v=onepage&q&f=false> [Accessed 9 June 2024].

Ji, F., Zhang, D., Shao, Y., Yu, X., Liu, X., Shan, D. and Wang, Z., 2020. Changes in the diversity and composition of gut microbiota in pigeon squabs infected with *Trichomonas gallinae*. *Scientific Reports*, [e-journal] 10(1), pp.19978. https://doi.org/10.1038/s41598-020-76821-9.

Jing, W., Liu, J., Wu, S., Li, X. and Liu, Y., 2021. Role of *cpxA* mutations in the resistance to aminoglycosides and β-lactams in *Salmonella enterica* serovar Typhimurium. *Frontiers in Microbiology*, [e-journal] 12, pp.604079. https://doi.org/10.3389/fmicb.2021.604079.

Johan, S.A., Abu Bakar, U., Mohd Taib, F.S. and Khairat, J.E., 2022. House crows (*Corvus splendens*): the carrier of pathogenic viruses or the misunderstood bird?. *Journal of Applied Animal Research*, [e-journal] 50(1), pp.678-686. https://doi.org/10.1080/09712119.2022.2133902.

Johne, R., Otto, P., Roth, B., Löhren, U., Belnap, D., Reetz, J. and Trojnar, E., 2011. Sequence analysis of the VP6-encoding genome segment of avian group F and G rotaviruses. *Virology*, [e-journal] 412(2), pp.384-391. https://doi.org/10.1016/j.virol.2011.01.031.

Johnson, M.D., Bell, J., Clarke, K., Chandler, R., Pathak, P., Xia, Y., Marshall, R.L., Weinstock, G.M., Loman, N.J., Winn, P.J. and Lund, P.A., 2014. Characterization of mutations in the PAS domain of the *EvgS* sensor kinase selected by laboratory evolution for acid resistance in *Escherichia coli*. *Molecular Microbiology*, [e-journal] 93(5), pp.911-927. https://doi.org/10.1111/mmi.12704.

Johnston, R.F. and Janiga, M., 1995. *Feral pigeons* (Vol. 4). [e-book] Oxford University Press on Demand. Available at: Google Books<https://books.google.com.my/books?hl=en&lr=&id=whScRZEmRl4C&oi=fnd&pg=PA3&dq=Johnston,+Richard+F.+Birds+of+North+America+No.+13,+1992.+The+American+Ornithologists%27+Union.&ots=eWxMtGK_7D&sig=KTOrp8FizUouVMTjPkZYlfXPtlo&redir_esc=y#v=onepage&q&f=false> [Accessed 30 July 2022].

Jokimäki, J. and Suhonen, J., 1998. Distribution and habitat selection of wintering birds in urban environments. *Landscape and Urban Planning*, [e-journal] 39(4), pp.253-263. https://doi.org/10.1016/S0169-2046(97)00089-3.

Jones, R.N., 2010. Microbial etiologies of hospital-acquired bacterial

pneumonia and ventilator-associated bacterial pneumonia. *Clinical Infectious Diseases*, [e-journal] 51(Supplement_1), pp.S81-S87. https://doi.org/10.1086/653053.

Juliao, P., Guzman-Holst, A., Gupta, V., Velez, C., Petrozzi, V. and Ochoa, T.J., 2021. Acute gastroenteritis morbidity and mortality trends following universal rotavirus vaccination in children in Peru: ecological database study with time-trend analysis. *Infectious Diseases and Therapy*, [e-journal] 10, pp.2563-2574. https://doi.org/10.1007/s40121-021-00532-5.

Kaczorek-Łukowska, E., Sowińska, P., Franaszek, A., Dziewulska, D., Małaczewska, J. and Stenzel, T., 2021. Can domestic pigeon be a potential carrier of zoonotic *Salmonella*?. *Transboundary and Emerging Diseases*, [e-journal] 68(4), pp.2321-2333. https://doi.org/10.1111/tbed.13891.

Kaeuffer, C., Baldacini, M., Ruge, T., Ruch, Y., Zhu, Y.J., De Cian, M., Philouze, G., Bachellier, P., Denis, J., Lefebvre, N. and Schneider, F., 2022. Fungal infections caused by *Kazachstania* spp., Strasbourg, France, 2007–2020. *Emerging Infectious Diseases*, [e-journal] 28(1), pp.30-35. https://doi.org/10.3201/eid2801.211543.

Kalantar, K.L., Carvalho, T., de Bourcy, C.F., Dimitrov, B., Dingle, G., Egger, R., Han, J., Holmes, O.B., Juan, Y.F., King, R. and Kislyuk, A., 2020. IDseq—An open source cloud-based pipeline and analysis service for metagenomic pathogen detection and monitoring. *Gigascience*, [e-journal] 9(10), pp.111. https://doi.org/10.1093/gigascience/giaa111.

Kamel, A.M., 2014. Potential impacts of invasive house crows (*Corvus splendens*) bird species in Ismailia Governorate, Egypt; ecology, control and risk management. *Journal of Life Sciences and Technologies*, [e-journal] 2(2), pp.86-89. https://doi.org/10.12720/jolst.2.2.86-89.

Kan, X.Z., Li, X.F., Zhang, L.Q., Chen, L., Qian, C.J., Zhang, X.W. and Wang, L., 2010. Characterization of the complete mitochondrial genome of the Rock pigeon, *Columba livia* (Columbiformes: *Columbidae*). *Genetics and Molecular Research*, [e-journal] 9(2), pp.1234-1249. https://doi.org/10.4238/vol9-2gmr853.

Kang, G., 2017. Viral diarrhea. *International Encyclopedia of Public Health*, [e-journal] 24, pp.360-367. https://doi.org/10.1016/B978-0-12-803678-5.00486-0.

Kapgate, S.S., Kumanan, K., Vijayarani, K. and Barbuddhe, S.B., 2018. Avian parvovirus: classification, phylogeny, pathogenesis and diagnosis. *Avian Pathology*, [e-journal] 47(6), pp.536-545. https://doi.org/10.1080/03079457.2018.1517938.

Kapikian, A.Z., 2001. A rotavirus vaccine for prevention of severe diarrhoea of infants and young children: development, utilization and withdrawal. *Gastroenteritis Viruses: Novartis Foundation Symposium* [e-journal] 238(238), pp.153-179. https://doi.org/10.1002/0470846534.ch10.

Kato, A., Ohnishi, H., Yamamoto, K., Furuta, E., Tanabe, H. and Utsumi, R., 2000. Transcription of *emrKY* is regulated by the *EvgA-EvgS* two-component system in *Escherichia coli* K-12. *Bioscience, Biotechnology, and Biochemistry*, [e-journal] 64(6), pp.1203-1209. https://doi.org/10.1271/bbb.64.1203.

Kazakova, J., Fernández-Torres, R., Ramos-Payán, M. and Bello-López, M.Á., 2018. Multiresidue determination of 21 pharmaceuticals in crayfish (*Procambarus clarkii*) using enzymatic microwave-assisted liquid extraction and ultrahigh-performance liquid chromatography-triple quadrupole mass spectrometry analysis. *Journal of Pharmaceutical and Biomedical Analysis*, [e-journal] 160, pp.144-151. https://doi.org/10.1016/j.jpba.2018.07.057.

Khalid, N., Bukhari, S.M., Ali, W. and Sheikh, A.A., 2023. Comparative Study on the Predominance of *Lactobacillus* spp. and *Escherichia Coli* in Healthy vs Colibacillosis Diseased Broilers. *Brazilian Journal of Poultry Science*, [e-journal] 25(03), pp.2022. https://doi.org/10.1590/1806-9061-2022-1758.

Khot, P.D. and Fisher, M.A., 2013. Novel approach for differentiating *Shigella* species and *Escherichia coli* by matrix-assisted laser desorption ionization–time of flight mass spectrometry. *Journal of Clinical Microbiology*, [e-journal] 51(11), pp.3711-3716. https://doi.org/10.1128/jcm.01526-13.

Khot, P.D., Couturier, M.R., Wilson, A., Croft, A. and Fisher, M.A., 2012. Optimization of matrix-assisted laser desorption ionization–time of flight mass spectrometry analysis for bacterial identification. *Journal of Clinical Microbiology*, [e-journal] 50(12), pp.3845-3852. https://doi.org/10.1128/jcm.00626-12.

Kim, C.Y., Oh, H., Song, J., Hur, M., Suh, J.H., Jheong, W.H., Kim, J.T., Oh, H.S. and Park, J.H., 2016. First detection of West Nile virus in domestic pigeon in Korea. *Journal of Veterinary Science*, [e-journal] 17(4), pp.587-589. https://doi.org/10.4142/jvs.2016.17.4.587.

Kohls, A., Lüschow, D., Lierz, M. and Hafez, H.M., 2011. Influenza A virus monitoring in urban and free-ranging pigeon populations in Germany, 2006–2008. *Avian Diseases*, [e-journal] 55(3), pp.447-450. https://doi.org/10.1637/9567-100710-ResNote.1.

Kombiah, S., Kumar, M., Murugkar, H.V., Nagarajan, S., Tosh, C., Kumar, D.S., Rajukumar, K., Gautam, S., Singh, R., Karikalan, M. and Sharma, A.K., 2020. Experimental pathology of two highly pathogenic H5N1 viruses isolated from crows in BALB/c mice. *Microbial Pathogenesis*, [e-journal] 141, pp.103984. https://doi.org/10.1016/j.micpath.2020.103984.

Kontoyiannis, D.P., Torres, H.A., Chagua, M., Hachem, R., Tarrand, J.J., Bodey, G.P. and Raad, I.I., 2004. Trichosporonosis in a tertiary care cancer center: risk factors, changing spectrum and determinants of outcome. *Scandinavian Journal of Infectious Diseases*, [e-journal] 36(8), pp.564-569. https://doi.org/10.1080/00365540410017563.

Korcari, D., Ricci, G., Capusoni, C. and Fortina, M.G., 2021. Physiological performance of *Kazachstania unispora* in sourdough environments. *World Journal of Microbiology and Biotechnology*, [e-journal] 37, pp.1-8. https://doi.org/10.1007/s11274-021-03027-0.

Koutserimpas, C., Samonis, G., Velivassakis, E., Iliopoulou-Kosmadaki, S., Kontakis, G. and Kofteridis, D.P., 2018. *Candida glabrata* prosthetic joint infection, successfully treated with anidulafungin: A case report and review of the literature. *Mycoses*, [e-journal] 61(4), pp.266- 269. https://doi.org/10.1111/myc.12736.

Kozdruń, W., Czekaj, H. and Styś, N., 2015. Avian zoonoses–a review. *Journal of Veterinary Research*, [e-journal] 59(2), pp.171-178. https://doi.org/10.1515/bvip-2015-0026.

Kratchenko, V., 2009. *Rock Doves or Rock Pigeons (Columba livia)*. [Online] Available at: <https://beautyofbirds.com/rock-pigeons/> [Accessed 17 November 2022].

Krause, K.M., Serio, A.W., Kane, T.R. and Connolly, L.E., 2016. Aminoglycosides: an overview. *Cold Spring Harbor Perspectives in Medicine*, [e-journal] 6(6), pp.027029. https://doi.org/10.1101/cshperspect.a027029.

Krzossok, S., Birck, R., Henke, S., Hof, H., van Der Woude, F.J. and Braun, C., 2004. *Trichosporon asahii* infection of a dialysis PTFE arteriovenous graft. *Clinical Nephrology*, [e-journal] 62(1), pp.66-68. https://doi.org/10.5414/cnp62066.

Kuhlmann, J., Dalhoff, A. and Zeiler, H.J. eds., 2012. *Quinolone antibacterials* (Vol. 127). [e-book] Heidelberg: Springer Berlin. Available at: Google Books < https://books.google.com.my/books?hl=en&lr=&id=eH_qCAAAQBAJ&oi=fnd&pg=PA1&dq=Quinolone+antibacterials+(Vol.+127).+&ots=1w16NNNeYh&sig=6HaOiXrmhhsa3G6WVAYR7QkBJR4&redir_esc=y#v=onepage&q=Quinolone%20antibacterials%20(Vol.%20127).&f=false> [Accessed 5 May 2024].

Kuiken, T., Buijs, P., van Run, P., van Amerongen, G., Koopmans, M. and van den Hoogen, B., 2017. Pigeon paramyxovirus type 1 from a fatal human case induces pneumonia in experimentally infected cynomolgus macaques (*Macaca fascicularis*). *Veterinary Research*, [e-journal] 48, pp.1-9. https://doi.org/10.1186/s13567-017-0486-6.

Kurtzman, C.P., Robnett, C.J., Ward, J.M., Brayton, C., Gorelick, P. and Walsh, T.J., 2005. Multigene phylogenetic analysis of pathogenic *Candida* species in the *Kazachstania (Arxiozyma) telluris* complex and description of their ascosporic states as *Kazachstania bovina* sp. nov., *K. heterogenica* sp. nov., *K. pintolopesii* sp. nov., and *K. slooffiae* sp. nov. *Journal of Clinical Microbiology*, [e-journal] 43(1), pp.101-111. https://doi.org/10.1128/jcm.43.1.101-111.2005

LaDeau, S.L., Calder, C.A., Doran, P.J. and Marra, P.P., 2011. West Nile virus impacts in American crow populations are associated with human land use and climate. *Ecological Research*, [e-journal] 26, pp.909-916. https://doi.org/10.1007/s11284-010-0725-z.

Lawson, B., De Pinna, E., Horton, R.A., Macgregor, S.K., John, S.K., Chantrey, J., Duff, J.P., Kirkwood, J.K., Simpson, V.R., Robinson, R.A. and Wain, J., 2014. Epidemiological evidence that garden birds are a source of human salmonellosis in England and Wales. *PloS One*, [e-journal] 9(2), pp.88968. https://doi.org/10.1371/journal.pone.0088968.

Le, V., Nhu, N.T.K., Cerdeno-Tarraga, A., Campbell, J.I., Tuyen, H.T., Nhu, T.D.H., Tam, P.T.T., Schultsz, C., Thwaites, G., Thomson, N.R. and Baker, S., 2015. Genetic characterization of three *qnrS1*-harbouring multidrug-resistance plasmids and *qnrS1*-containing transposons circulating in Ho Chi Minh City, Vietnam. *Journal of Medical Microbiology*, [e-journal] 64(8), pp.869-878. https://doi.org/10.1099/jmm.0.000100.

Lee, W.D., Fong, J.J., Eimes, J.A. and Lim, Y.W., 2017. Diversity and abundance of human-pathogenic fungi associated with pigeon faeces in urban environments. *Molecular Ecology*, [e-journal] 26(17), pp.4574-4585. https://doi.org/10.1111/mec.14216.

Lefebvre, L. and Giraldeau, L.A., 1984. Daily feeding site use of urban pigeons. *Canadian Journal of Zoology*, [e-journal] 62(7), pp.1425-1428. https://doi.org/10.1139/z84-204.

Lema, N.K., Gemeda, M.T. and Woldesemayat, A.A., 2023. Recent advances in metagenomic approaches, applications, and challenges. *Current Microbiology*, [e-journal] 80(11), pp.347. https://doi.org/10.1007/s00284-023-03451-5.

Li, H., Guo, M., Wang, C., Li, Y., Fernandez, A.M., Ferraro, T.N., Yang, R. and Chen, Y., 2020. Epidemiological study of *Trichosporon asahii* infections over the past 23 years. *Epidemiology and Infection*, [e-journal] *148*, pp.169. https://doi.org/10.1017/S0950268820001624.

Li, K.S., Guan, Y., Wang, J., Smith, G.J.D., Xu, K.M., Duan, L., Rahardjo, A.P., Puthavathana, P., Buranathai, C., Nguyen, T.D. and Estoepangestie, A.T.S., 2004. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. *Nature*, [e-journal] 430(6996), pp.209-213. https://doi.org/10.1038/nature02746.

Li, X., Wang, S., Li, W., Wang, S., Qin, X., Wang, J. and Fu, R., 2024. Investigating pigeon circovirus infection in a pigeon farm: molecular detection, phylogenetic analysis and complete genome analysis. *BMC Genomics*, [e-journal] 25(1), pp.369. https://doi.org/10.1186/s12864-024-10303-4.

Limtong, S., Yongmanitchai, W., Tun, M.M., Kawasaki, H. and Seki, T., 2007. *Kazachstania siamensis* sp. nov., an ascomycetous yeast species from forest soil in Thailand. *International Journal of Systematic and Evolutionary*

Microbiology, [e-journal] 57(2), pp.419-422. https://doi.org/10.1099/ijs.0.64539-0.

Lin, Y., Zhang, L., Wu, J. and Yang, K., 2023. Wild birds-the sentinel of antibiotic resistance for urban river: Study on egrets and Jinjiang river in Chengdu, China. *Environmental Research*, [e-journal] 216, pp.114566. https://doi.org/10.1016/j.envres.2022.114566.

Ling, Y., Chen, H., Chen, X., Yang, X., Yang, J., Bavoil, P.M. and He, C., 2015. Epidemiology of *Chlamydia psittaci* infection in racing pigeons and pigeon fanciers in Beijing, China. *Zoonoses and Public Health*, [e-journal] 62(5), pp.401-406. https://doi.org/10.1111/zph.12161.

Liu, F., Hu, Z.D., Yurkov, A., Chen, X.H., Bao, W.J., Ma, Q., Zhao, W.N., Pan, S., Zhao, X.M., Liu, J.H. and Wang, Q.M., 2023. *Saccharomycetaceae*: delineation of fungal genera based on phylogenomic analyses, genomic relatedness indices and genomics-based synapomorphies. *Persoonia-Molecular Phylogeny and Evolution of Fungi*. [e-journal] 52, pp.1-21. https://doi.org/10.3767/persoonia.2024.52.01.

Liu, S., Luo, H., Wang, M., Wang, Q., Duan, L., Han, Q., Sun, S., Wei, C. and Jin, J., 2022. Microbiome analysis reveals the effects of black soldier fly oil on gut microbiota in pigeon. *Frontiers in Microbiology*, [e-journal] 13, pp.998524. https://doi.org/10.3389/fmicb.2022.998524.

Loiko, M.R., Junqueira, D.M., Varela, A.P.M., Tochetto, C., Scheffer, C.M., Lima, D.A., Morel, A.P., Cerva, C., Paim, W.P., Mayer, F.Q. and Roehe, P.M., 2018. Columbid circoviruses detected in free ranging pigeons from Southern Brazil: insights on PiCV evolution. *Archives of Virology*, [e-journal] 163, pp.3083-3090. https://doi.org/10.1007/s00705-018-3990-8.

Lomovskaya, O., Lewis, K.I.M. and Matin, A., 1995. EmrR is a negative regulator of the *Escherichia coli* multidrug resistance pump EmrAB. *Journal of Bacteriology*, [e-journal] 177(9), pp.2328-2334. https://doi.org/10.1128/jb.177.9.2328-2334.1995.

Lorrot, M. and Vasseur, M., 2007. How do the rotavirus NSP4 and bacterial enterotoxins lead differently to diarrhea?. *Virology Journal*, [e-journal] 4(31), pp.1-6. https://doi.org/10.1186/1743-422X-4-31.

Lu, D., Kalantar, K.L., Chu, V.T., Glascock, A.L., Guerrero, E.S., Bernick, N., Butcher, X., Ewing, K., Fahsbender, E., Holmes, O. and Hoops, E., 2024. Simultaneous detection of pathogens and antimicrobial resistance genes with the open source, cloud-based, CZ ID pipeline. *BioRxiv*. [e-journal] pp.589250. https://doi.org/10.1101/2024.04.12.589250.

Łukaszuk, E. and Stenzel, T., 2020. Occurrence and Role of selected RNA-viruses as potential causative agents of watery droppings in pigeons. *Pathogens*, [e-journal] 9(12), pp.1025. https://doi.org/10.3390/pathogens9121025.

Łukaszuk, E., Dziewulska, D. and Stenzel, T., 2024. Rotaviruses in pigeons with diarrhea: recovery of three complete pigeon rotavirus A genomes and the first case of pigeon rotavirus G in Europe. *Transboundary and Emerging Diseases*, [e-journal] 2024(1), pp.4684235. https://doi.org/10.1155/tbed/4684235.

Mair-Jenkins, J., Lamming, T., Dziadosz, A., Flecknoe, D., Stubington, T., Mentasti, M., Muir, P. and Monk, P., 2018. A psittacosis outbreak among english office workers with little or no contact with birds, August 2015. *PLoS Currents*, [e-journal] 10. https://doi.org/10.1371/currents.outbreaks.b646c3bb2b4f0e3397183f31823bbca6.

Makinodan, K., Yoshikawa, M., Fukuoka, A., Yoshimoto, E., Tamaki, S., Tomoda, K., Hamada, K. and Kimura, H., 2005. A familial case of summer-type hypersensitivity pneumonitis possibly associated with bird breeder's lung diagnosed by bronchoalveolar lavage fluid. *Nihon Kokyuki Gakkai zasshi= the journal of the Japanese Respiratory Society*, 43(11), pp.693-699.

Maldonado, Y., Fiser, J.C., Nakatsu, C.H. and Bhunia, A.K., 2005. Cytotoxicity potential and genotypic characterization of *Escherichia coli* isolates from environmental and food sources. *Applied and Environmental Microbiology*, [e-journal] 71(4), pp.1890-1898. https://doi.org/10.1128/AEM.71.4.1890-1898.2005.

Mankertz, A., Hattermann, K., Ehlers, B. and Soike, D., 2000. Cloning and sequencing of columbid circovirus (CoCV), a new circovirus from pigeons. *Archives of Virology*, [e-journal] 145, pp.2469-2479. https://doi.org/10.1007/s007050070002.

Mansour, S.M., ElBakrey, R.M., Ali, H., Knudsen, D.E. and Eid, A.A., 2014. Natural infection with highly pathogenic avian influenza virus H5N1 in domestic pigeons (*Columba livia*) in Egypt. *Avian Pathology*, [e-journal] 43(4), pp.319-324. https://doi.org/10.1080/03079457.2014.926002.

Martella, V., Bányai, K., Matthijnssens, J., Buonavoglia, C. and Ciarlet, M., 2010. Zoonotic aspects of rotaviruses. *Veterinary Microbiology*, [e-journal] 140(3-4), pp.246-255. https://doi.org/10.1016/j.vetmic.2009.08.028.

Martinson, J.N. and Walk, S.T., 2020. *Escherichia coli* residency in the gut of healthy human adults. *EcoSal Plus*, [e-journal] 9(1), pp.10-1128. https://doi.org/10.1128/ecosalplus.esp-0003-2020.

Martín-Vélez, V., Lovas-Kiss, Á., Sánchez, M.I. and Green, A.J., 2021. Endozoochory of the same community of plants lacking fleshy fruits by storks and gulls. *Journal of Vegetation Science*, [e-journal] 32(1), pp.12967. https://doi.org/10.1111/jvs.12967.

Matthijnssens, J., Attoui, H., Bányai, K., Brussaard, C.P., Danthi, P., Del Vas, M., Dermody, T.S., Duncan, R., Fāng, Q., Johne, R. and Mertens, P.P., 2022. ICTV virus taxonomy profile: *Sedoreoviridae* 2022. *Journal of General Virology*, [e-journal] 103(10), pp.001782. https://doi.org/10.1099/jgv.0.001782.

Matthijnssens, J., Ciarlet, M., McDonald, S.M., Attoui, H., Bányai, K., Brister, J.R., Buesa, J., Esona, M.D., Estes, M.K., Gentsch, J.R. and Iturriza-Gómara, M., 2011. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). *Archives of Virology*, [e-journal] 156, pp.1397-1413. https://doi.org/10.1007/s00705-011-1006-z.

Matthijnssens, J., Ciarlet, M., Rahman, M., Attoui, H., Bányai, K., Estes, M.K., Gentsch, J.R., Iturriza-Gómara, M., Kirkwood, C.D., Martella, V. and Mertens, P.P., 2008. Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. *Archives of Virology*, [e-journal] 153, pp.1621-1629. https://doi.org/10.1007/s00705-008-0155-1.

Matthijnssens, J., Rahman, M., Martella, V., Xuelei, Y., De Vos, S., De Leener, K., Ciarlet, M., Buonavoglia, C. and Van Ranst, M., 2006. Full genomic analysis of human rotavirus strain B4106 and lapine rotavirus strain 30/96 provides evidence for interspecies transmission. *Journal of Virology*, [e-journal] 80(8), pp.3801-3810. https://doi.org/10.1128/jvi.80.8.3801-3810.2006.

Mazurek-Popczyk, J. and Baldy-Chudzik, K., 2021. Differentiation of Trimethoprim Resistance Genes among *Eschericha coli* Strains from an Environment with Intensive Supply of Antibiotics. S*ci Forum*, [e-journal] pp.8-17. https://doi.org/10.3390/ECA2021-09648.

McCowan, C., Crameri, S., Kocak, A., Shan, S., Fegan, M., Forshaw, D., Rubbenstroth, D., Chen, H., Holmes, C., Harper, J. and Dearnley, M., 2018. A novel group A rotavirus associated with acute illness and hepatic necrosis in pigeons (*Columba livia*), in Australia. *PloS One*, [e-journal] 13(9), pp.0203853. https://doi.org/10.1371/journal.pone.0203853.

McNabb, F.A., McNabb, R.A. and Steeves III, H.R., 1973. Renal mucoid materials in pigeons fed high and low protein diets. *The Auk*, [e-journal] 90(1), pp.14-18. https://doi.org/10.1093/auk/90.1.14.

Medina, I.R., Fuentes, L.R., Arteaga, M.B., Valcárcel, F.R., Arbelo, F.A., Del Castillo, D.P., Suárez, S.D., Quintana, O.F., Gutiérrez, B.V., Sergent, F.S. and Acosta-Hernández, B., 2017. Pigeons and their droppings as reservoirs of *Candida* and other zoonotic yeasts. *Revista Iberoamericana de Micologia*, [e-journal] 34(4), pp.211-214. https://doi.org/10.1016/j.riam.2017.03.001.

Mehta, V., Nayyar, C., Gulati, N., Singla, N., Rai, S. and Chandar, J., 2021. A comprehensive review of *Trichosporon* spp.: an invasive and emerging fungus. *Cureus*, [e-journal] 13(8), pp.17345. https://doi.org/10.7759/cureus.17345.

Meßmer, C., Rubbenstroth, D., Mohr, L., Peus, E., Schreiber, T. and Rautenschlein, S., 2022. Pigeon Rotavirus A as the cause of systemic infection in juvenile pigeons (young pigeon disease). *Tierärztliche Praxis Ausgabe K: Kleintiere/Heimtiere*, [e-journal] 50(04), pp.293-301. https://doi.org/10.1055/a-1909-2235.

Mihalov-Kovács, E., Gellért, Á., Marton, S., Farkas, S. L., Fehér, E., Oldal, M.,

Jakab, F., Martella, Vito., Bányai, K., 2015. Candidate New Rotavirus Species in Sheltered Dogs, Hungary. *Emerging Infectious Diseases*, [e-journal] 21(4), 660-663. https://doi.org/10.3201/eid2104.141370.

Minamoto, N., Oki, K., Tomita, M., Kinjo, T., Suzuki, Y., 1988. Isolation and characterization of rotavirus from feral pigeon in mammalian cell cultures. *Epidemiology and Infection*. [e-journal] 100(3), pp.481–492. https://doi.org/10.1017/S0950268800067212.

Miranda, A., Ávila, B., Díaz, P., Rivas, L., Bravo, K., Astudillo, J., Bueno, C., Ulloa, M.T., Hermosilla, G., Del Canto, F. and Salazar, J.C., 2016. Emergence of plasmid-borne *dfrA14* trimethoprim resistance gene in *Shigella sonnei*. *Frontiers in Cellular and Infection Microbiology*, [e-journal] 6, pp.77. https://doi.org/10.3389/fcimb.2016.00077.

Monaco, F., Lelli, R., Teodori, L., Pinoni, C., Di Gennaro, A., Polci, A., Calistri, P. and Savini, G., 2010. Re-emergence of West Nile virus in Italy. *Zoonoses and Public Health*, [e-journal] 57(7-8), pp.476-486. https://doi.org/10.1111/j.1863-2378.2009.01245.x.

Montanari, G., Zambonelli, C., Grazia, L., Kamesheva, G.K. and Shigaeva, M.K., 1996. *Saccharomyces unisporus* as the principal alcoholic fermentation microorganism of traditional koumiss. *Journal of Dairy Research*, [e-journal] 63(2), pp.327-331. https://doi.org/10.1017/S0022029900031836.

Morabito, S., Dell'Omo, G., Agrimi, U., Schmidt, H., Karch, H., Cheasty, T. and Caprioli, A., 2001. Detection and characterization of Shiga toxin-producing *Escherichia coli* in feral pigeons. *Veterinary Microbiology*, [e-journal] 82(3), pp.275-283. https://doi.org/10.1016/s0378-1135(01)00393-5.

Morell, F., Roger, À., Reyes, L., Cruz, M.J., Murio, C. and Muñoz, X., 2008. Bird fancier's lung: a series of 86 patients. *Medicine*, [e-journal] 87(2), pp.110-130. https://doi.org/10.1097/MD.0b013e31816d1dda.

Mori, Y., Sugiyama, M., Takayama, M., Atoji, Y., Masegi, T. and Minamoto, N., 2001. Avian-to-mammal transmission of an avian rotavirus: analysis of its pathogenicity in a heterologous mouse model. *Virology*, [e-journal] 288(1), pp.63-70. https://doi.org/10.1006/viro.2001.1051.

Motteu, N., Goemaere, B., Bladt, S. and Packeu, A., 2022. Implementation of MALDI-TOF mass spectrometry to identify fungi from the indoor environment as an added value to the classical morphology-based identification tool. *Frontiers in Allergy*, [e-journal] 3, pp.826148. https://doi.org/10.3389/falgy.2022.826148.

Moulin, G., Cavalié, P., Pellanne, I., Chevance, A., Laval, A., Millemann, Y., Colin, P. and Chauvin, C., 2008. A comparison of antimicrobial usage in human and veterinary medicine in France from 1999 to 2005. *Journal of Antimicrobial Chemotherapy*, [e-journal] 62(3), pp.617-625. https://doi.org/10.1093/jac/dkn213.

Mueller, H. and Johne, R., 2007. Rotaviruses: diversity and zoonotic potential--a brief review. *Berliner und Munchener Tierarztliche Wochenschrift*, 120(3-4), pp.108-112.

Mundy, L.M., Sahm, D.F. and Gilmore, M., 2000. Relationships between enterococcal virulence and antimicrobial resistance. *Clinical Microbiology Reviews*, [e-journal] 13(4), pp.513-522. https://doi.org/10.1128/cmr.13.4.513.

Murton, R.K. and Westwood, N.J., 1966. The foods of the rock dove and feral pigeon. *Bird Study*, [e-journal] 13(2), pp.130-146. https://doi.org/10.1080/00063656609476116.

Myhealty Ministry of Health Malaysia, 2024. *Rotavirus Vaccine*. [online] Available at: <http://www.myhealth.gov.my/en/rotavirus-Vaccine/#:~:text=In%20Malaysia%2C%20about%2022%25%20to,children%20are%20caused%20by%20rotavirus.&text=Rotavirus%20spreads%20easily%20from%20hand,their%20own%20from%20the%20infection.> [Accessed 15 October 2024].

Nath, B.K., Das, T., Peters, A., Gupta, S.D., Sarker, S., Forwood, J.K., Raidal, S.R. and Das, S., 2023. Australasian Pigeon Circoviruses Demonstrate Natural Spillover Infection. *Viruses*, [e-journal] 15(10), pp.2025. https://doi.org/10.3390/v15102025.

National Notifiable Diseases Surveillance System, 2024. *Nationally Notifiable Infectious Diseases and Conditions, United States: Weekly Tables*. [online] Available at: <https://wonder.cdc.gov//nndss/static/2024/24/2024-24-table1020.html> [Accessed 10 November 2024].

Naz, S.A., Yaseen, M., Jabeen, N. and Shafique, M., 2017. Isolation of potentially pathogenic fungi from selected pigeons' feeding sites in Karachi: A new dimension to health hazard. *Journal of the Pakistan Medical Association*, [e-journal] 67(6), pp.901-906.

Neumann, G., Chen, H., Gao, G.F., Shu, Y. and Kawaoka, Y., 2010. H5N1 influenza viruses: outbreaks and biological properties. *Cell Research*, [e-journal] 20(1), pp.51-61. https://doi.org/10.1038/cr.2009.124.

Nishino, K. and Yamaguchi, A., 2002. EvgA of the two-component signal transduction system modulates production of the yhiUV multidrug transporter in *Escherichia coli*. *Journal of Bacteriology*, [e-journal] 184(8), pp.2319-2323. https://doi.org/10.1128/jb.184.8.2319-2323.2002.

Nishino, K. and Yamaguchi, A., 2004. Role of histone-like protein H-NS in multidrug resistance of *Escherichia coli*. *Journal of Bacteriology*, [e-journal] 186(5), pp.1423-1429. https://doi.org/10.1128/jb.186.5.1423-1429.2004.

Nkosi, B.V.Z., Padayachee, T., Gront, D., Nelson, D.R. and Syed, K., 2022. Contrasting health effects of Bacteroidetes and Firmicutes lies in their genomes: Analysis of P450s, ferredoxins, and secondary metabolite clusters. *International Journal of Molecular Sciences*, [e-journal] 23(9),

pp.5057. https://doi.org/10.3390/ijms23095057.

Noverr, M.C., Cox, G.M., Perfect, J.R. and Huffnagle, G.B., 2003. Role of *PLB1* in pulmonary inflammation and cryptococcal eicosanoid production. *Infection and Immunity*, [e-journal] 71(3), pp.1538-1547. https://doi.org/10.1128/iai.71.3.1538-1547.2003.

Nualmalang, R., Thanomsridetchai, N., Teethaisong, Y., Sukphopetch, P. and Tangwattanachuleeporn, M., 2023. Identification of pathogenic and opportunistic yeasts in pigeon excreta by MALDI-TOF mass spectrometry and their prevalence in Chon Buri province, Thailand. *International Journal of Environmental Research and Public Health*, [e-journal] 20(4), pp.3191. https://doi.org/10.3390/ijerph20043191.

O'Brien, V.A., Meteyer, C.U., Reisen, W.K., Ip, H.S. and Brown, C.R., 2010. Prevalence and pathology of West Nile virus in naturally infected house sparrows, western Nebraska, 2008. *The American Journal of Tropical Medicine and Hygiene*, [e-journal] 82(5), pp.937-944. https://doi.org/10.4269/ajtmh.2010.09-0515.

Oh, S., Park, S.H., Choi, J.H., Kim, S.L., Kim, M., Lee, S., Yi, M.H., Lee, I.Y., Yong, T.S. and Kim, J.Y., 2023. The microbiota in feces of domestic pigeons in Seoul, Korea. *Heliyon*, [e-journal] 9(4), pp14997. https://doi.org/10.1016/j.heliyon.2023.e14997.

Osman, K.M., Mehrez, M., Erfan, A.M. and Nayerah, A., 2013. *Salmonella enterica* isolated from pigeon (*Columba livia*) in Egypt. *Foodborne Pathogens and Disease*, [e-journal] 10(5), pp.481-483. https://doi.org/10.1089/fpd.2012.1347.

Ovo control, 2018. *How fast do pigeons reproduce.* [Online] Available at: <https://www.ovocontrol.com/news-blog/2018/01/how-fast-do-pigeons-reproduce> [Accessed 31 July 2022].

Padilla-Vaca, F., Mondragon-Jaimes, V. and Franco, B., 2017. General aspects of two-component regulatory circuits in bacteria: domains, signals and roles. *Current Protein and Peptide Science*, [e-journal] 18(10), pp.990-1004. https://doi.org/10.2174/1389203717666160809154809.

Pakshir, K., Zareshahrabadi, Z., Zomorodian, K., Ansari, S., Nouraei, H. and Gharavi, A., 2019. Molecular identification of non-*Cryptococcus* yeasts associated with pigeon droppings in Shiraz, Southern Iran. *Iranian Journal of Veterinary Research*, *20*(3), p.204-208.

Pandey, N. and Cascella, M., 2019. Beta lactam antibiotics. [StatPearls] Treasure Island (FL): StatPearls. Available at: Ncbi.nlm.nih.gov <https://www.ncbi.nlm.nih.gov/sites/books/NBK545311/> [Accessed 4 May 2024].

Pasmans, F., Baert, K., Martel, A., Bousquet-Melou, A., Lanckriet, R., De Boever, S., Van Immerseel, F., Eeckhaut, V., De Backer, P. and Haesebrouck,

F., 2008. Induction of the carrier state in pigeons infected with *Salmonella enterica* subspecies *enterica* serovar typhimurium PT99 by treatment with florfenicol: a matter of pharmacokinetics. *Antimicrobial Agents and Chemotherapy*, [e-journal] 52(3), pp.954-961. https://doi.org/10.1128/aac.00575-07.

Pasmans, F., Van Immerseel, F., Hermans, K., Heyndrickx, M., Collard, J.M., Ducatelle, R. and Haesebrouck, F., 2004. Assessment of virulence of pigeon isolates of *Salmonella enterica* subsp. *enterica* serovar Typhimurium variant Copenhagen for humans. *Journal of Clinical Microbiology*, [e-journal] 42(5), pp.2000-2002. https://doi.org/10.1128/JCM.42.5.2000-2002.2004.

Paul, T.K., Amin, M.R., Alam, M.A., Rahman, M.K., Sarker, Y.A. and Rizon, M.K., 2015. Occurrence of pigeon diseases at Khulna Sadar, Bangladesh. *Bangladesh Journals of Veternary Medicine*, [e-journal] 13(2), pp.21-25. https://doi.org/10.3329/bjvm.v13i2.26616.

Pauly, M., Oni, O.O., Sausy, A., Owoade, A.A., Adeyefa, C.A., Muller, C.P., Hübschen, J.M. and Snoeck, C.J., 2017. Molecular epidemiology of Avian Rotaviruses Group A and D shed by different bird species in Nigeria. *Virology Journal*, [e-journal] 14, pp.1-10. https://doi.org/10.1186/s12985-017-0778-5.

Pedersen, K., Clark, L., Andelt, W.F. and Salman, M.D., 2006. Prevalence of Shiga toxin– producing *Escherichia coli* and *Salmonella enterica* in rock pigeons captured in Fort Collins, Colorado. *Journal of Wildlife Diseases*, [e-journal] 42(1), pp.46-55. https://doi.org/10.7589/0090-3558-42.1.46.

Pérez-Vazquez, M., Oteo-Iglesias, J., Sola-Campoy, P.J., Carrizo-Manzoni, H., Bautista, V., Lara, N., Aracil, B., Alhambra, A., Martínez-Martínez, L., Campos, J. and Spanish Antibiotic Resistance Surveillance Program Collaborating Group, 2019. Characterization of carbapenemase-producing *Klebsiella oxytoca* in Spain, 2016–2017. *Antimicrobial Agents and Chemotherapy*, [e-journal] 63(6), pp.10-1128. https://doi.org/10.1128/aac.02529-18.

Phan, T.G., Vo, N.P., Boros, A., Pankovics, P., Reuter, G., Li, O.T., Wang, C., Deng, X., Poon, L.L. and Delwart, E., 2013. The viruses of wild pigeon droppings. *PloS One*, [e-journal] 8(9), pp.72787. https://doi.org/10.1371/journal.pone.0072787.

Piddock, L.J., 2006. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clinical Microbiology Reviews*, [e-journal] 19(2), pp.382-402. https://doi.org/10.1128/cmr.19.2.382-402.2006.

Pigeon Control Resource Centre, 2009. *Pigeons - everything there is to know about the pigeon.* [Online] Available at: <https://www.pigeoncontrolresourcecentre.org/html/about-pigeons.html#topofpage> [Accessed 5 August 2022].

Podschun, R. and Ullmann, U., 1998. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clinical Microbiology Reviews*, [e-journal] 11(4), pp.589-603.

https://doi.org/10.1128/cmr.11.4.589.

Poetranto, E.D., Yamaoka, M., Nastri, A.M., Krisna, L.A.W., Rahman, M.H., Wulandari, L., Yudhawati, R., Ginting, T.E., Makino, A., Shinya, K. and Kawaoka, Y., 2011. An H5N1 highly pathogenic avian influenza virus isolated from a local tree sparrow in Indonesia. *Microbiology and Immunology*, [e-journal] 55(9), pp.666-672. https://doi.org/10.1111/j.1348-0421.2011.00361.x.

Powlesland, R.G., 2013. *Rock pigeon | kererū aropari*. [online] Available at: <https://www.nzbirdsonline.org.nz/species/rock-pigeon> [Accessed 5 May 2024].

Prince, D.S., Astry, C., Vonderfecht, S., JAKAB, G., SHEN, F.M. and Yolken, R.H., 1986. Aerosol transmission of experimental rotavirus infection. *The Pediatric Infectious Disease Journal*, [e-journal] 5(2), pp.218-222. https://doi.org/10.1097/00006454-198603000-00012.

Pritchard, L.I., Chua, K.B., Cummins, D., Hyatt, A., Crameri, G., Eaton, B.T. and Wang, L.F., 2006. Pulau virus; a new member of the Nelson Bay orthoreovirus species isolated from fruit bats in Malaysia. *Archives of Virology*, [e-journal] 151, pp.229-239. https://doi.org/10.1007/s00705-005-0644-4.

Puzari, M., Sharma, M. and Chetia, P., 2018. Emergence of antibiotic resistant *Shigella* species: A matter of concern. *Journal of Infection and Public Health*, [e-journal] 11(4), pp.451-454. https://doi.org/10.1016/j.jiph.2017.09.025.

Queiroz-Aaltonen, I.R.D.O., Melo, M.F.D., Fonseca, L.A.V.D., Silva, D.M.W. and Maranhão, F.C.A., 2021. Molecular detection of medically important *Candida* species from droppings of pigeons (*Columbiformes*) and captive birds (*Passeriformes* and *Psittaciformes*). *Brazilian Archives of Biology and Technology*, [e-journal] 64, pp.21200763. https://doi.org/10.1590/1678-4324-2021200763.

Rabsch, W., Andrews, H.L., Kingsley, R.A., Prager, R., Tschäpe, H., Adams, L.G. and Bäumler, A.J., 2002. *Salmonella enterica* serotype Typhimurium and its host-adapted variants. *Infection and Immunity*, [e-journal] 70(5), pp.2249-2255. https://doi.org/10.1128/iai.70.5.2249-2255.2002.

Radimersky, T., Frolkova, P., Janoszowská, D., Dolejská, M., Svec, P., Roubalová, E., Cikova, P., Cizek, A. and Literák, I., 2010. Antibiotic resistance in faecal bacteria (*Escherichia coli*, *Enterococcus* spp.) in feral pigeons. *Journal of Applied Microbiology*, [e-journal] 109(5), pp.1687-1695. https://doi.org/10.1111/j.1365-2672.2010.04797.x.

Rådström, P., Swedberg, G.O.T.E. and Sköld, O., 1991. Genetic analyses of sulfonamide resistance and its dissemination in gram-negative bacteria illustrate new aspects of R plasmid evolution. *Antimicrobial Agents and Chemotherapy*, [e-journal] 35(9), pp.1840-1848. https://doi.org/10.1128/aac.35.9.1840.

Rahman, M., Mangtani, P., Uyeki, T.M., Cardwell, J.M., Torremorell, M., Islam, A., Samad, M.A., Muraduzzaman, A.K.M., Giasuddin, M., Sarkar, S. and Alamgir, A.S.M., 2020. Evaluation of potential risk of transmission of avian influenza A viruses at live bird markets in response to unusual crow die-offs in Bangladesh. *Influenza and Other Respiratory Viruses*, [e-journal] 14(3), pp.349-352. https://doi.org/10.1111/irv.12716.

Ramachandran, V.L.A.K.P.G.S.G.A.V., 1999. *Enterobacter aerogenes* outbreak in a neonatal intensive care unit. *Pediatrics International*, [e-journal] 41(2), pp.157-161. https://doi.org/10.1046/j.1442-200X.1999.4121033.x.

Ramig, R.F., 1997. Genetics of the rotaviruses. *Annual Review of Microbiology*, [e-journal] 51(1), pp.225-255. https://doi.org/10.1146/annurev.micro.51.1.225.

Ramig, R.F., 2007. Systemic rotavirus infection. *Expert Review of Anti-infective Therapy*, [e-journal] 5(4), pp.591-612. https://doi.org/10.1586/14787210.5.4.591.

Ranjbar, R. and Farahani, A., 2019. *Shigella*: antibiotic-resistance mechanisms and new horizons for treatment. *Infection and Drug Resistance*, [e-journal] pp.3137-3167. https://doi.org/10.2147/IDR.S219755.

Raue, R., Schmidt, V., Freick, M., Reinhardt, B., Johne, R., Kamphausen, L., Kaleta, E.F., Müller, H. and Krautwald-Junghanns, M.E., 2005. A disease complex associated with pigeon circovirus infection, young pigeon disease syndrome. *Avian Pathology*, [e-journal] 34(5), pp.418-425. https://doi.org/10.1080/03079450500267825.

Razmyar, J., Movassaghi, A., Mirshahi, A., Eidi, S., Zaeemi, M. and Rezaee, M., 2014. Osteoarthritis and systemic infection caused by *Candida Albicans* in a common Mynah (*Acridotheres tristis*). *Iranian Journal of Veterinary Science and Technology*, [e-journal] 6(2), pp.77-84. https://doi.org/10.22067/veterinary.v6i2.27859.

Reed, C.E., Sosman, A. and Barbee, R.A., 1965. Pigeon-breeders' lung: a newly observed interstitial pulmonary disease. *Jama*, [e-journal] 193(4), pp.261-265. https://doi.org/10.1001/jama.1965.03090040005001.

Reyes-Fernández, E.Z., Cudkowicz, N.A., Steiner-Mordoch, S. and Schuldiner, S., 2021. Identification of *Escherichia coli* Genes Essential for High-Level, Clinically Relevant, Resistance to Antibiotics. *BioRxiv*, [e-journal] pp.2021-11. https://doi.org/10.1101/2021.11.10.468170.

Reygaert, W.C., 2018. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*, [e-journal] 4(3), pp.482-501. https://doi.org/10.3934/microbiol.2018.3.482.

Rice, L.B., Carias, L., Rudin, S., Vael, C., Goossens, H., Konstabel, C., Klare, I., Nallapareddy, S.R., Huang, W. and Murray, B.E., 2003. A potential virulence gene, hyl_{Efm}, predominates in *Enterococcus faecium* of clinical origin. *The*

Journal of Infectious Diseases, [e-journal] 187(3), pp.508-512. https://doi.org/10.1086/367711.

Rinninella, E., Cintoni, M., Raoul, P., Lopetuso, L.R., Scaldaferri, F., Pulcini, G., Miggiano, G.A.D., Gasbarrini, A. and Mele, M.C., 2019. Food components and dietary habits: keys for a healthy gut microbiota composition. *Nutrients*, [e-journal] 11(10), pp.2393. https://doi.org/10.3390/nu11102393.

Rocha-e-Silva, R.C., Cardoso, W.M., Teixeira, R.S.C., Albuquerque, Á.H., Horn, R.V., Lopes, E.S., Gomes Filho, V.J.R., Almeida, C.P., Santos, I.C.L., Machado, D.N. and Lima, S.V.G., 2014. Isolation of *Salmonella enterica* subsp. enterica (O: 4, 5: i) and *Salmonella enterica* subsp. Typhimurium from free-living domestic pigeons (*Columba livia*). *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, [e-journal] 66(5), pp.1435-1438. https://doi.org/10.1590/1678-6716.

Rodgers, D., Le, C., Pimentel, C., Tuttobene, M.R., Subils, T., Escalante, J., Nishimura, B., Vescovi, E.G., Sieira, R., Bonomo, R.A. and Tolmasky, M.E., 2021. Histone-like nucleoid-structuring protein (H-NS) regulatory role in antibiotic resistance in *Acinetobacter baumannii*. *Scientific Reports*, [e-journal] 11(1), pp.18414. https://doi.org/10.1038/s41598-021-98101-w.

Rohwedder, A., Schütz, K.I., Minamoto, N. and Brüssow, H., 1995. Sequence analysis of pigeon, turkey, and chicken rotavirus VP8* identifies rotavirus 993/83, isolated from calf feces, as a pigeon rotavirus. *Virology*, [e-journal] 210(1), pp.231-235. https://doi.org/10.1006/viro.1995.1338.

Roossinck, M.J., 2016. *Virus: An Illustrated Guide to 101 Incredible Microbes*. [e-book] Princeton University Press. Available at: Google Books < https://books.google.com.my/books?hl=en&lr=&id=gTH9CwAAQBAJ&oi=fnd&pg=PA10&dq=Roossinck,+M.J.,+2016.+Virus:+An+Illustrated+Guide+to+101+Incredible+Microbes.+Princeton+University+Press.&ots=2HMLVBXiXt&sig=ChQXJExz0Ji1v49DAUODXHn73HA&redir_esc=y#v=onepage&q&f=false> [Accessed 6 June 2024].

Rosario, I., Soro, G., Deniz, S., Ferrer, O., Acosta, F., Padilla, D. and Acosta, B., 2010. Presence of *C. albidus*, *C. laurentii* and *C. uniguttulatus* in crop and droppings of pigeon lofts (*Columba livia*). *Mycopathologia*, [e-journal] 169, pp.315-319. https://doi.org/10.1007/s11046-009-9262-0.

Ross, H., 2022. *What do pigeons eat?*. [Online] Available at: <https://a-zanimals.com/blog/what-do-pigeons-eat/> [Accessed 16 November 2022].

Ruan, S.Y., Chien, J.Y. and Hsueh, P.R., 2009. Invasive trichosporonosis caused by *Trichosporon asahii* and other unusual *Trichosporon* species at a medical center in Taiwan. *Clinical Infectious Diseases*, [e-journal] 49(1), pp.11-e17. https://doi.org/10.1086/599614.

Rubbenstroth, D., Peus, E., Schramm, E., Kottmann, D., Bartels, H., McCowan, C., Schulze, C., Akimkin, V., Fischer, N., Wylezich, C. and Hlinak, A., 2019. Identification of a novel clade of group A rotaviruses in fatally diseased

domestic pigeons in Europe. *Transboundary and Emerging Diseases*, [e-journal] 66(1), pp.552-561. https://doi.org/10.1111/tbed.13065.

Rubbenstroth, D., Ulrich, R., Wylezich, C., Rautenschlein, S., Beer, M. and Mohr, L., 2020. First experimental proof of Rotavirus A (RVA) genotype G18P [17] inducing the clinical presentation of 'young pigeon disease syndrome'(YPDS) in domestic pigeons (*Columba livia*). *Transboundary and Emerging Diseases*, [e-journal] 67(4), pp.1507-1516. https://doi.org/10.1111/tbed.13485.

Rudkjøbing, V.B., Thomsen, T.R., Xu, Y., Melton-Kreft, R., Ahmed, A., Eickhardt, S., Bjarnsholt, T., Poulsen, S.S., Nielsen, P.H., Earl, J.P. and Ehrlich, G.D., 2016. Comparing culture and molecular methods for the identification of microorganisms involved in necrotizing soft tissue infections. *BMC Infectious Diseases*, [e-journal] 16(1), pp.652. https://doi.org/10.1186/s12879-016-1976-2.

Sabin, A.B., 1959. Reoviruses: a new group of respiratory and enteric viruses formerly classified as ECHO type 10 is described. *Science*, [e-journal] 130(3386), pp.1387-1389. https://doi.org/10.1126/science.130.3386.1387.

Sachse K, Laroucau K, Riege K, Wehner S, Dilcher M, Creasy HH, Weidmann M, Myers G, Vorimore F, Vicari N, et al. 2014. Evidence for the existence of two new members of the family *Chlamydiaceae* and proposal of *Chlamydia avium* sp. nov. and *Chlamydia gallinacea* sp. nov. *Systematic and Applied Microbiology*. [e-journal] 37, pp.79–88. https://doi.org/10.1016/j.syapm.2013.12.004.

Sánchez-Osuna, M., Cortés, P., Barbé, J. and Erill, I., 2019. Origin of the mobile di-hydro-pteroate synthase gene determining sulfonamide resistance in clinical isolates. *Frontiers in Microbiology*, [e-journal] 9, pp.3332. https://doi.org/10.3389/fmicb.2018.03332.

Santos, H.M., Tsai, C.Y., Catulin, G.E.M., Trangia, K.C.G., Tayo, L.L., Liu, H.J. and Chuang, K.P., 2020. Common bacterial, viral, and parasitic diseases in pigeons (*Columba livia*): A review of diagnostic and treatment strategies. *Veterinary Microbiology*, [e-journal] 247, pp.108779. https://doi.org/10.1016/j.vetmic.2020.108779.

Schmidt, V., Kümpel, M., Cramer, K., Sieg, M., Harzer, M., Rückner, A. and Heenemann, K., 2021. Pigeon rotavirus A genotype G18P [17]-associated disease outbreaks after fancy pigeon shows in Germany–a case series. *Tierärztliche Praxis Ausgabe K: Kleintiere/Heimtiere*, [e-journal] 49(01), pp.22-27. https://doi.org/10.1055/a-1339-0366.

Schwaber, M.J., Klarfeld-Lidji, S., Navon-Venezia, S., Schwartz, D., Leavitt, A. and Carmeli, Y., 2008. Predictors of carbapenem-resistant *Klebsiella pneumoniae* acquisition among hospitalized adults and effect of acquisition on mortality. *Antimicrobial Agents and Chemotherapy*, [e-journal] 52(3), pp.1028-1033. https://doi.org/10.1128/aac.01020-07.

Servin, A.L., 2004. Antagonistic activities of *lactobacilli* and *bifidobacteria*

against microbial pathogens. *FEMS Microbiology Reviews*, [e-journal] 28(4), pp.405-440. https://doi.org/10.1016/j.femsre.2004.01.003.

Shaiber, A. and Eren, A.M., 2019. Composite metagenome-assembled genomes reduce the quality of public genome repositories. *MBio*, [e-journal] 10(3), pp.10-1128. https://doi.org/10.1128/mBio.00725-19.

Sharma, P., Haycocks, J.R., Middlemiss, A.D., Kettles, R.A., Sellars, L.E., Ricci, V., Piddock, L.J. and Grainger, D.C., 2017. The multiple antibiotic resistance operon of enteric bacteria controls DNA repair and outer membrane integrity. *Nature Communications*, [e-journal] 8(1), pp.1444. https://doi.org/10.1038/s41467-017-01405-7.

Shen, Y., Xiao, W.Q., Gong, J.M., Pan, J. and Xu, Q.X., 2017. Detection of New Delhi Metallo-Beta-Lactamase (Encoded by bla_{NDM-1}) in *Enterobacter aerogenes* in China. *Journal of Clinical Laboratory Analysis*, [e-journal] 31(2), pp.22044. https://doi.org/10.1002/jcla.22044.

Shin, N.R., Whon, T.W. and Bae, J.W., 2015. Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends in Biotechnology*, [e-journal] 33(9), pp.496-503. https://doi.org/10.1016/j.tibtech.2015.06.011.

Shirai, T., Furusawa, H., Furukawa, A., Ishige, Y., Uchida, K., Miyazaki, Y., Eishi, Y. and Inase, N., 2017. Protein antigen of bird-related hypersensitivity pneumonitis in pigeon serum and dropping. *Respiratory Research*, [e-journal] 18, pp.1-9. https://doi.org/10.1186/s12931-017-0555-4.

Silva, V.L., Nicoli, J.R., Nascimento, T.C. and Diniz, C.G., 2009. Diarrheagenic *Escherichia coli* strains recovered from urban pigeons (*Columba livia*) in Brazil and their antimicrobial susceptibility patterns. *Current Microbiology*, [e-journal] 59(3), pp.302-308. https://doi.org/10.1007/s00284-009-9434-7.

Singh, L., Cariappa, M.P. and Kaur, M., 2016. *Klebsiella oxytoca*: An emerging pathogen?. *Medical Journal Armed Forces India*, [e-journal] 72, pp.S59-S61. https://doi.org/10.1016/j.mjafi.2016.05.002.

Singhal, N., Kumar, M., Kanaujia, P.K. and Virdi, J.S., 2015. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Frontiers in Microbiology*, [e-journal] 6, pp.791. https://doi.org/10.3389/fmicb.2015.00791.

Sirhandi, B.A., Shaikh, K., Rashidi, S.F., Shabrani, W.A., Arain, I., Arain, M., Gachal, G.S., Raees, I., Rajput, K.S. and Shaikh, A.R., 2021. Diversity, ecology and conservation of pigeons and doves (Family *Columbidae*) in Pakistan. *International Journal of Ecosystems and Ecology Science*, [e-journal] 11(2), pp.355-360. https://doi.org/10.31407/ijees11.223.

Sköld, O., 2001. Resistance to trimethoprim and sulfonamides. *Veterinary Research*, [e-journal] 32(3-4), pp.261-273. https://doi.org/10.1051/vetres:2001123.

Soh, M.C., Pang, R.Y., Ng, B.X., Lee, B.P.H., Loo, A.H., Er, K.B., 2021. Restricted human activities shift the foraging strategies of feral pigeons (*Columba livia*) and three other commensal bird species. *Biological Conservation*, [e-journal] 253, pp.108927. https://doi.org/10.1016/j.biocon.2020.108927.

Soltani, M., Bayat, M., Hashemi, S.J., Zia, M. and Pestechian, N., 2013. *Isolation of Cryptococcus neoformans and other opportunistic fungi from pigeon droppings.* [online] Available at: < https://pubmed.ncbi.nlm.nih.gov/23901339/#:~:text=The%20frequency%20rate%20of%20C, risk%20of%20contracting%20opportunistic%20diseases> [Accessed 17 November 2022].

Spennemann, D.H. and Watson, M.J., 2017. Dietary habits of urban pigeons (*Columba livia*) and implications of excreta pH–a review. *European Journal of Ecology*, [e-journal] 3(1), pp.27-41. https://doi.org/10.1515/eje-2017-0004.

Spennemann, D.H., Pike, M. and Watson, M.J., 2017. Effects of acid pigeon excreta on building conservation. *International Journal of Building Pathology and Adaptation*, [e-journal] 35(1), pp.2-15. https://doi.org/10.1108/IJBPA-09-2016-0023.

Srikanta, D., Santiago-Tirado, F.H. and Doering, T.L., 2014. *Cryptococcus neoformans*: historical curiosity to modern pathogen. *Yeast*, [e-journal] 31(2), pp.47-60. https://doi.org/10.1002/yea.2997.

Stenzel, T. and Koncicki, A., 2017. The epidemiology, molecular characterization and clinical pathology of circovirus infections in pigeons–current knowledge. *Veterinary Quarterly*, [e-journal] 37(1), pp.166-174. https://doi.org/10.1080/01652176.2017.1325972.

Stenzel, T. and Pestka, D., 2014. Occurrence and genetic diversity of pigeon circovirus strains in Poland. *Acta Veterinaria Hungarica*, [e-journal] 62(2), pp.274-283. https://doi.org/10.1556/avet.2014.004.

Stenzel, T., Dziewulska, D., Tykałowski, B. and Koncicki, A., 2020. The clinical infection with pigeon circovirus (PiCV) leads to lymphocyte B apoptosis but has no effect on lymphocyte T subpopulation. *Pathogens*, [e-journal] 9(8), pp.632. https://doi.org/10.3390/pathogens9080632.

Stock, B. and Haag-Wackernagel, D., 2016. Food shortage affects reproduction of Feral Pigeons (*Columba livia*) at rearing of nestlings. *International Journal of Avian Science*, [e-journal] 158(4), pp.776-783. https://doi.org/10.1111/ibi.12385.

Struthers, J.D., 2024. Doves and Pigeons. *Pathology of Pet and Aviary Birds*, [e-journal] pp.481-512. https://doi.org/10.1002/9781119650522.ch15.

Stukenholtz, E.E., Hailu, T.A., Childers, S., Leatherwood, C., Evans, L., Roulain, D., Townsley, D., Treider, M., Platt, R.N., Ray, D.A. and Zak, J.C., 2019. *Ecology of feral pigeons: Population monitoring, resource selection, and*

management practices. [e-book] USA: IntechOpen. Available at: < https://www.intechopen.com/chapters/66330> [Accessed 15 November 2022].

Sugita, T., Nishikawa, A., Ichikawa, T., Ikeda, R. and Shinoda, T., 2000. Isolation of *Trichosporon asahii* from environmental materials. *Medical Mycology*, [e-journal] 38(1), pp.27-30. https://doi.org/10.1080/mmy.38.1.27.30.

Svensson, L., Sheshberadaran, H., Vesikari, T., Norrby, E. and Wadell, G., 1987. Immune response to rotavirus polypeptides after vaccination with heterologous rotavirus vaccines (RIT 4237, RRV-1). *Journal of General Virology*, [e-journal] 68(7), pp.1993-1999. https://doi.org/10.1099/0022-1317-68-7-1993.

Svraka, S., Rosario, K., Duizer, E., van der Avoort, H., Breitbart, M. and Koopmans, M., 2010. Metagenomic sequencing for virus identification in a public-health setting. *Journal of General Virology*, [e-journal] 91(11), pp.2846-2856. https://doi.org/10.1099/vir.0.024612-0.

Szymańska-Czerwińska, M. and Niemczuk, K., 2016. Avian chlamydiosis zoonotic disease. *Vector-Borne and Zoonotic Diseases*, [e-journal] 16(1), pp.1-3. https://doi.org/10.1089/vbz.2015.1839.

Tanaka, C., Miyazawa, T., Watarai, M. and Ishiguro, N., 2005. Bacteriological survey of feces from feral pigeons in Japan. *Journal of Veterinary Medical Science*, [e-journal] 67(9), pp.951-953. https://doi.org/10.1292/jvms.67.951.

Taras, L., Kubíček, O., Juranova, R. and Jurajda, V., 2003. The first demonstration of pigeon circovirus infection in the Czech Republic based on histological testing and nested PCR. *Acta Veterinaria Brno*, *72*(4), pp.577-582.

Tavío, M.M., Jacoby, G.A. and Hooper, D.C., 2014. *QnrS1* structure–activity relationships. *Journal of Antimicrobial Chemotherapy*, [e-journal] 69(8), pp.2102-2109. https://doi.org/10.1093/jac/dku102.

Tiwari, S., Jamal, S.B., Hassan, S.S., Carvalho, P.V., Almeida, S., Barh, D., Ghosh, P., Silva, A., Castro, T.L. and Azevedo, V., 2017. Two-component signal transduction systems of pathogenic bacteria as targets for antimicrobial therapy: an overview. *Frontiers in Microbiology*, [e-journal] 8, pp.1878. https://doi.org/10.3389/fmicb.2017.01878.

Todd, D., Duchatel, J.P., Bustin, J.C., Scullion, F.T., Scullion, M.G., Scott, A.N.J., Curry, A., Ball, N.W. and Smyth, J.A., 2006. Detection of pigeon circovirus in cloacal swabs: implications for diagnosis, epidemiology and control. *Veterinary Record*, [e-journal] 159(10), pp.314-317. https://doi.org/10.1136/vr.159.10.314.

Universiti Tunku Abdul Rahman (UTAR), 2024. *UTAR Map*. [online] Available at: <https://dsa.kpr.utar.edu.my/documents/UTARMap.jpg> [Accessed 5 June 2024].

Vallejo Timarán, D.A., Benavides Melo, C.J., Chaves Velásquez, C.A., Morillo Caicedo, M.I. and Castillo Ceballos, A.M., 2016. *Cryptococcus neoformans*

isolation in pigeon (*columba livia*) feces in the urban area of the municipality of Pasto, Colombia. *Biosalud*, [e-journal] 15(1), pp.62-71. https://doi.org/10.17151/biosa.2016.15.1.7.

Van Duin, D., Kaye, K.S., Neuner, E.A. and Bonomo, R.A., 2013. Carbapenem-resistant *Enterobacteriaceae*: a review of treatment and outcomes. *Diagnostic Microbiology and Infectious Disease*, [e-journal] 75(2), pp.115-120. https://doi.org/10.17151/biosa.2016.15.1.7.

van Lent S, Piet JR, Beeckman D, van der Ende A, Van Nieuwerburgh F, Bavoil P, Myers G, Vanrompay D, Pannekoek Y. 2012. Full genome sequences of all nine *Chlamydia psittaci* genotype reference strains. *Journal of Bacteriology*, [e-journal] 194, pp.6930–6931. https://doi.org/10.1128/jb.01828-12.

Vasconcellos, H.V.G.D., Silva, K.F.B., Montenegro, H., Miguel, C.B., Tizioto, P., Agostinho, F., Araújo, M.C., Ribas, R.M., Silva, M.V.D., Soares, S.D.C. and Rodrigues, V., 2022. *Staphylococcus aureus* and *Enterococcus faecium* isolated from pigeon droppings (*Columba livia*) in the external environment close to hospitals. *Revista da Sociedade Brasileira de Medicina Tropical*, [e-journal] 55, pp.0353-2021. https://doi.org/10.1590/0037-8682-0353-2021.

Vashishtha, V.M., Mittal, A. and Garg, A., 2012. A fatal outbreak of *Trichosporon asahii* Sepsis in a neonatal intensive care unit. *Indian Pediatrics*, [e-journal] 49, pp.745-747. https://doi.org/10.1007/s13312-012-0137-y.

Vázquez, B., Esperón, F., Neves, E., López, J., Ballesteros, C. and Muñoz, M.J., 2010. Screening for several potential pathogens in feral pigeons (*Columba livia*) in Madrid. *Acta Veterinaria Scandinavica*, [e-journal] 52(45), pp.1-6. https://doi.org/10.1186/1751-0147-52-45.

Venturi, M., Guerrini, S. and Vincenzini, M., 2012. Stable and non-competitive association of *Saccharomyces cerevisiae*, *Candida milleri* and *Lactobacillus sanfranciscensis* during manufacture of two traditional sourdough baked goods. *Food Microbiology*, [e-journal] 31(1), pp.107-115. https://doi.org/10.1016/j.fm.2012.02.011.

Vlahović, K., Matica, B., Bata, I., Pavlak, M., Pavičić, Ž., Popović, M., Nejedli, S. and Dovč, A., 2004. *Campylobacter, Salmonella* and *Chlamydia* in free-living birds of Croatia. *European Journal of Wildlife Research*, [e-journal] 50(3), pp.127-132. https://doi.org/10.1007/s10344-004-0052-1.

Vogel, J. and Papenfort, K., 2006. Small non-coding RNAs and the bacterial outer membrane. *Current Opinion in Microbiology*, [e-journal] 9(6), pp.605-611. https://doi.org/10.1016/j.mib.2006.10.006.

Vučemilo, M., Vlahović, K., Dovč, A., MuŽinić, J., Pavlak, M., Jerčić, J. and Župančić, Ž., 2003. Prevalence of *Campylobacter jejuni*, *Salmonella Typhimurium*, and avian Paramyxovirus type 1 (PMV-1) in pigeons from different regions in Croatia. *Zeitschrift für Jagdwissenschaft*, [e-journal] 49, pp.303-313. https://doi.org/10.1007/BF02189638.

Walsh, C., Owens, R.A., Bottacini, F., Lane, J.A., van Sinderen, D. and Hickey, R.M., 2023. HMO-primed *bifidobacteria* exhibit enhanced ability to adhere to intestinal epithelial cells. *Frontiers in Microbiology*, [e-journal] 14, pp.1232173. https://doi.org/10.3389/fmicb.2023.1232173.

Walter, J.E. and Yee, R.B., 1968. Factors that determine the growth of *Cryptococcus neoformans* in avian excreta. *American Journal of Epidemiology*, [e-journal] 88(3), pp.445- 450. https://doi.org/10.1093/oxfordjournals.aje.a120906.

Wang, H., Gao, H., Jiang, Z., Shi, L., Zhao, P., Zhang, Y. and Wang, C., 2022. Molecular detection and phylogenetic analysis of pigeon circovirus from racing pigeons in Northern China. *BMC Genomics*, [e-journal] 23(1), pp.290. https://doi.org/10.1186/s12864-022-08425-8.

Wang, J., Ma, Z.B., Zeng, Z.L., Yang, X.W., Huang, Y. and Liu, J.H., 2017a. The role of wildlife (wild birds) in the global transmission of antimicrobial resistance genes. *Zoological Research*, [e-journal] 38(2), pp.55-80. https://doi.org/10.24272/j.issn.2095-8137.2017.003.

Wang, K.C., Zhuang, Q.Y., Qiu, Y., Wang, T. and Chen, J.M., 2017b. Genome sequence characterization of pigeon circoviruses in China. *Virus Research*, [e-journal] 233, pp.1-7. https://doi.org/10.1016/j.virusres.2017.03.007.

Wani, S.A., Samanta, I., Bhat, M.A. and Nishikawa, Y., 2004. Investigation of shiga toxin- producing *Escherichia coli* in avian species in India. *Letters in Applied Microbiology*, [e-journal] 39(5), pp.389-394. https://doi.org/10.1111/j.1472-765X.2004.01586.x.

Warscheid, T. and Braams, J., 2000. Biodeterioration of stone: a review. *International Biodeterioration & Biodegradation*, [e-journal] 46(4), pp.343-368. https://doi.org/10.1016/S0964-8305(00)00109-8.

Waseem, H., Williams, M.R., Stedtfeld, T., Chai, B., Stedtfeld, R.D., Cole, J.R., Tiedje, J.M. and Hashsham, S.A., 2017. Virulence factor activity relationships (VFARs): a bioinformatics perspective. *Environmental Science: Processes & Impacts*, [e-journal] 19(3), pp.247-260. https://doi.org/10.1039/c6em00689b.

Weatherspoon-Griffin, N., Yang, D., Kong, W., Hua, Z. and Shi, Y., 2014. The CpxR/CpxA two-component regulatory system up-regulates the multidrug resistance cascade to facilitate *Escherichia coli* resistance to a model antimicrobial peptide. *Journal of Biological Chemistry*, [e-journal] 289(47), pp.32571-32582. https://doi.org/10.1074/jbc.M114.565762.

Werner, O., Römer-Oberdörfer, A., Köllner, B., Manvell, R.J. and Alexander, D.J., 1999. Characterization of avian paramyxovirus type 1 strains isolated in Germany during 1992 to 1996. *Avian Pathology*, [e-journal] 28(1), pp.79-88. https://doi.org/10.1080/03079459995082.

Wille, M., Shi, M., Hurt, A.C., Klaassen, M. and Holmes, E.C., 2021. RNA virome abundance and diversity is associated with host age in a bird

species. *Virology*, [e-journal] 561, pp.98-106. https://doi.org/10.1016/j.virol.2021.06.007.

Wirth, F. and Goldani, L.Z., 2012. Epidemiology of *Rhodotorula*: an emerging pathogen. *Interdisciplinary Perspectives on Infectious Diseases,* [e-journal] 2012, pp.1-7. https://doi.org/10.1155/2012/465717.

World Health Organization (WHO), 2020. *Campylobacter*. [Online] Available at: < https://www.who.int/news-room/fact-sheets/detail/campylobacter> [Accessed 15 November 2022].

World Health Organization (WHO), 2024. *Human infection with avian influenza A (H5) viruses: Human infection with avian influenza A (H5N1) virus, Avian influenza weekly update number 970.* [online] Available at: < https://cdn.who.int/media/docs/default-source/wpro---documents/emergency/surveillance/avian-influenza/ai_20241025.pdf?sfvrsn=5f006f99_143#:~:text=From%201%20January%202003%20to,(CFR)%20of%2054%25.> [Accessed 13 October 2024].

Wu, C., Zheng, L. and Yao, J., 2022. Analysis of risk factors and mortality of patients with carbapenem-resistant *Klebsiella pneumoniae* infection. *Infection and Drug Resistance,* [e-journal] 15, pp.2383-2391. https://doi.org/10.2147/IDR.S362723.

Wu, J., Huang, Y., Rao, D., Zhang, Y. and Yang, K., 2018. Evidence for environmental dissemination of antibiotic resistance mediated by wild birds. *Frontiers in Microbiology*, [e-journal] 9, pp.745. https://doi.org/10.3389/fmicb.2018.00745.

Wu, Y., Du, P.C., Li, W.G. and Lu, J.X., 2012. Identification and molecular analysis of pathogenic yeasts in droppings of domestic pigeons in Beijing, China. *Mycopathologia,* [e-journal] 174(3), pp.203-214. https://doi.org/10.1007/s11046-012-9536-9.

Xi, Y., Shuling, N., Kunyuan, T., Qiuyang, Z., Hewen, D., ChenCheng, G., Tianhe, Y., Liancheng, L. and Xin, F., 2019. Characteristics of the intestinal flora of specific pathogen free chickens with age. *Microbial Pathogenesis*, [e-journal] 132, pp.325-334. https://doi.org/10.1016/j.micpath.2019.05.014.

Xiong, A., Gottman, A., Park, C., Baetens, M., Pandza, S. and Matin, A., 2000. The *EmrR* protein represses the *Escherichia coli emrRAB* multidrug resistance operon by directly binding to its promoter region. *Antimicrobial Agents and Chemotherapy*, [e-journal] 44(10), pp.2905-2907. https://doi.org/10.1128/aac.44.10.2905-2907.2000.

Xu, Q., Zhao, W., Li, Y., Zou, X. and Dong, X., 2022. Intestinal immune development is accompanied by temporal deviation in microbiota composition of newly hatched pigeon squabs. *Microbiology Spectrum*, [e-journal] *10*(3), pp.01892-21. https://doi.org/10.1128/spectrum.01892-21.

Yamamoto, E., Ito, H., Kitamoto, E., Morinishi, K., Yano, A., Miyoshi, S. and

Ito, T., 2015. Complete genome sequence of pigeon circovirus detected in racing pigeons in western Japan. *Virus Genes*, [e-journal] 51, pp.140-143. https://doi.org/10.1007/s11262-015-1211-z.

Yan, R., Lu, M., Zhang, L., Yao, J., Li, S. and Jiang, Y., 2022. Effect of sex on the gut microbiota characteristics of passerine migratory birds. *Frontiers in Microbiology*, [e-journal] 13, pP.917373. https://doi.org/10.3389/fmicb.2022.917373.

Yao, W., Chen, X., Wu, Z., Wang, L., Shi, G., Yang, Z., Zhang, Y. and Wu, B., 2022. A cluster of Psittacosis cases in Lishui, Zhejiang Province, China, in 2021. *Frontiers in Cellular and Infection Microbiology*, [e-journal] 12, pP.1044984. Https://doi.org/10.3389/fcimb.2022.1044984.

Yinda, C.K., Ghogomu, S.M., Conceicao-Neto, N., Beller, L., Deboutte, W., Vanhulle, E., Maes, P., Van Ranst, M. and Matthijnssens, J., 2018. Cameroonian fruit bats harbor divergent viruses, including rotavirus H, bastroviruses, and picobirnaviruses using an alternative genetic code. *Virus Evolution*, [e-journal] 4(1), pp.008. https://doi.org/10.1093/ve/vey008.

Zafar, H. and Saier Jr, M.H., 2021. Gut Bacteroides species in health and disease. *Gut Microbes*, [e-journal] 13(1), pp.1848158. https://doi.org/10.1080/19490976.2020.1848158.

Zaidi, S.E.Z., Zaheer, R., Zovoilis, A. and McAllister, T.A., 2024. *Enterococci* as a One Health indicator of antimicrobial resistance. *Canadian Journal of Microbiology*, [e-journal] 70(8), pp.303-335. https://doi.org/10.1139/cjm-2024-0024.

Zell, R., Delwart, E., Gorbalenya, A.E., Hovi, T., King, A.M.Q., Knowles, N.J., Lindberg, A.M., Pallansch, M.A., Palmenberg, A.C., Reuter, G. and Simmonds, P., 2017. ICTV virus taxonomy profile: *Picornaviridae*. *Journal of General Virology*, [e-journal] 98(10), pp.2421-2422. https://doi.org/10.1099/jgv.0.000911.

Zhang, R. and Wang, Y., 2023. EvgS/EvgA, the unorthodox two-component system regulating bacterial multiple resistance. *Applied and Environmental Microbiology*, [e-journal] 89(12), pp.01577-23. https://doi.org/10.1128/aem.01577-23.

Zhang, S., Li, X., Wu, J., Coin, L., O'brien, J., Hai, F. and Jiang, G., 2021. Molecular methods for pathogenic bacteria detection and recent advances in wastewater analysis. *Water*, [e-journal] 13(24), pp.3551. https://doi.org/10.3390/w13243551.

Zhang, Z., Dai, W., Wang, S. and Dai, D., 2015. Epidemiology and genetic characteristics of pigeon circovirus (PiCV) in eastern China. *Archives of Virology*, [e-journal] 160, pp.199-206. https://doi.org/10.1007/s00705-014-2255-4.

Zhu, Z., Huang, Z., Li, Z., Li, X., Du, C. and Tian, Y., 2018. Multiple brain

abscesses caused by infection with *Candida glabrata*: A case report. *Experimental and Therapeutic Medicine*, [e-journal] 15(3), pp.2374-2380. https://doi.org/10.3892/etm.2018.5692.

APPENDICES A

The chemicals and reagents used in this study are listed in Table S1, while the equipments used in this study are listed in Table S2.

Table S1: List of chemicals and reagents involved in this study.

Chemical and reagent	Manufacturer	Producer's Country
Agarose powder	Vivantis	Malaysia
Tris Base	Fisher BioReagents	United States
EDTA	Alfa Aesar	United States
PEG 6000	Bio Basic Inc.	Canada
DNase	Vivantis	Malaysia
RNase	Vivantis	Malaysia
VC 1 kb DNA ladder	Vivantis	Malaysia
Molecular grade absolute ethanol	Merck	Germany
DNase & RNase free water	Bio Basic Inc.	Canada
Acetic acid glacial	QRëC	United States
ViSafe Red gel stain	Vivantis	Malaysia

Table S2: List of equipments involved in this study.

Equipment	Model	Manufacturer	Country of Origin
Microcentrifuge machine	Microfuge 16	Beckman Coulter®	United States
Vortex mixer	ZX3	VELP Scientifica	Italy
Gel imager	Universal Hood II	BIO-RAD	United States
Heat block	BSH1002-E	Benchmark Scientific	United States
Nanodrop	Nanodrop 2000	ThermoScientific™	United States
pH meter	Five Easy F20	Mettler Toledo	Switzerland
Water Bath	WTB50	Memmert GmbH	Germany
Power supply	BPS-2	BIOBASE	China

APPENDICES B

Table S3 Reference sequences of Pigeon Circovirus (PiCV) from the NCBI GenBank database for viral consensus genome assembly and phylogenetic analysis.

Pigeon Circovirus Strain	Accession number	Length	Location	Host
CoCV/Poland/PL53/2011/KF738860*	KF738860	2035	Poland	*Columba livia*
PiCV/China/XJ1/2018/MT614188*	MT614188	2031	China	*Columbidae*
PiCV/China/PiCV-LY8/2023/PP556374*	PP556374	2037	China	*Columbidae*
PiCV/China/GS-DN2023-1-3/2023/PP405066*	PP405066	2034	China: Gansu	*Columbidae*
PiCV/Poland/BIV_3a_C10/2022/OR801850*	OR801850	2034	Poland	*Columba livia*
PiCV/Poland/BIV_3a_C1/2022/OR801841*	OR801841	2034	Poland	*Columba livia*
PiCV/Poland/BIV_3a_C3/2022/OR801843*	OR801843	2034	Poland	*Columba livia*
PiCV/China/PiCVLY1/2023/PP556373*	PP556373	2037	China	*Columbidae*
PiCV/China/GX1/2023/OR941416*	OR941416	2035	China	*Columbidae*
PiCV/China/LQ1/SN/2018/MW181983*	MW181983	2035	China	*Columbidae*
PiCV/China/G2798/GuangDong/2014/KX108786*	KX108786	2037	China	*Columbidae*
PiCV/China/GF45/GuangDong/2014/KX108823*	KX108823	2031	China	*Columbidae*
PiCV/China/JS15-1/2015/KX431143*	KX431143	2034	China	*Columba*
PiCV/Belgium/11-08304/2011/JX901128*	JX901128	2033	Belgium	*Columba*
CoCV/Belgium/Bel20/DQ915958*	DQ915958	2032	Belgium	*Columbidae*

Table S3: (cont'd)

Pigeon Circovirus Strain	Accession number	Length	Location	Host
CoCV/RefSeq/USA/NC002361	NC_002361	2037	USA	*Columbidae*
PiCV/Poland/SI_1a_C5/2022/OR999200	OR999200	2038	Poland	*Columba livia*
PiCV/Poland/BIV_3a_C5/2022/OR801845	OR801845	2034	Poland	*Columba livia*
PiCV/Poland/BIV_3a_C8/2022/OR801848	OR801848	2035	Poland	*Columba livia*
PiCV/Canada/CDVUM-2022-28072747/2022/PP663502	PP663502	2043	Canada	*Corvus corax*
PiCV/China/HB-DN2023-4-1/2023/PP405068	PP405068	2037	China: Hebei	*Columbidae*
PiCV/China/SX-DN2023-6-1/2023/PP405070	PP405070	2040	China: Shanxi	*Columbidae*
PiCV/China/SX01/Shanxi/2023/PP301888	PP301888	2038	China	*Columbidae*
PiCV/Italy/ITA/2019/cat/518.1a/2019/OQ357580	OQ357580	2043	Italy	*Felis catus*
PiCV/China/QY2/HB/2022/OR250484	OR250484	2035	China	*Columbidae*
PiCV/Australia/PiCV-Senegal_Dove-CS19-1715/2019/MZ447864	MZ447864	2039	Australia	*Columbidae*
PiCV/China/BJ01/Beijing/2019/MW289588	MW289588	2034	China	*Columba*
PiCV/China/CA4/SN/2018/MW181955	MW181955	2035	China	*Columbidae*
PiCV/China/WL1/SN/2018/MW181956	MW181956	2042	China	*Columbidae*
PiCV/Poland/TSP19_2/2020/MW656002	MW656002	2034	Poland	*Columba livia*
PiCV/Poland/TSP19_3/2020/MW656003	MW656003	2034	Poland	*Columba livia*
PiCV/China/nm-szw6/2015/MN920392	MN920392	2042	China: Inner Mongolia	*Columbidae*

Table S3: (cont'd)

Pigeon Circovirus Strain	Accession number	Length	Location	Host
PiCV/Australia/PiCV/P02/AUS/2013/MF136680	MF136680	2037	Australia	*Columba livia*
PiCV/Australia/PiCV/P03/AUS/2013/MF136681	MF136681	2039	Australia	*Columba livia*
PiCV/Australia/PiCV/P08/AUS/2013/MF136684	MF136684	2037	Australia	*Columba livia*
PiCV/Australia/PiCV/P10/AUS/2013/MF136686	MF136686	2037	Australia	*Columba livia*
CoCV/Brazil/PR1625/2014/KX808543	KX808543	2041	Brazil	*Columba livia*
CoCV/Brazil/RS0120/2014/KY114965	KY114965	2041	Brazil	*Columba livia*
CoCV/Brazil/POA-MA2/2016/MF664481	MF664481	2038	Brazil	*Columba livia*
PiCV/Japan/2/2010/LC035390	LC035390	2041	Japan: Kagawa	*Columba livia*
CoCV/Poland/PL57/2011/KF738861	KF738861	2037	Poland	*Columba*
CoCV/Poland/PL58/2002/KF738862	KF738862	2038	Poland	*Columba livia*
PiCV/Belgium/98-324/1998/JX901125	JX901125	2037	Belgium	*Columba*
CoCV/China/fj1/2009/JN183455	JN183455	2037	China	*Columbidae*
CoCV/USA/SRK/US/01/2003/EU840176	EU840176	2041	USA	*Columba livia*
CoCV/Italy/Ita4B/DQ915950	DQ915950	2040	Italy	*Columbidae*
CoCV/Australia/Dove/DQ915959	DQ915959	2039	Australia	*Columbidae*
CoCV/France/FraA40042/DQ915960	DQ915960	2037	France	*Columbidae*
CoCV/USA/US93A/DQ915961	DQ915961	2037	USA	*Columbidae*

Table S3: (cont'd)

Pigeon Circovirus Strain	Accession number	Length	Location	Host
CoCV/USA/US002180/DQ915962	DQ915962	2037	USA	*Columbidae*
CoCV/UK/9030/AJ298229	AJ298229	2037	United Kingdom: Northern Ireland	*Columbidae*

**NCBI reference sequences selected for viral consensus genome assembly using CZ ID platform.*

Table S4: Reference sequences of Rotavirus A from the NCBI GenBank database for viral consensus genome assembly and phylogenetic analysis.

Rotavirus A Strain	Accession numbers	Location	Host
RVA/Pigeon-wt/USA/WVL21015-FL/2020/G18P[17]*	OK185344 - OK185354	USA	*Columba livia*
RVA/Pigeon-wt/Germany/GK-684/2017/G18P[17]*	MH568756 - MH568766	Germany	*Columba livia*
RVA/Pigeon/Australia/VIC/2016/G18P[17]*	MH668302 - MH668312	Australia	*Columbidae*
RVA/Pigeon-wt/China/SX-05/2019/G18P[17]*	MN481630 - MN481640	China	*Columba*
RVA/Pigeon-wt/Germany/DR-5/2016/G18P[17]*	MH568745 - MH568755	Germany	*Columba livia*
RVA/Fox-wt/Itali/288356/2011/G18P[17]*	KT873803 - KT873813	Italy	*Canidae*
RVA/VelvetScoter-tc/Japan/RK1/1989/G18P[17]*	LC088096 - LC088106	Japan	*Melanitta fusca*
RVA/Columbidae/USA/K1802315/2018/G18P[17]*	MK692877 - MK692887	USA	*Columbidae*
RVA/SpottedDove/China/ARO/2014/G18P[17]*	KT934648 - KT934658	China	*Spilopelia chinensis*
AvianRotavirus/Pigeon-tc/Japan/PO-13/1983/G18P[17]*	AB009625 - AB009633, D82979, D16329	Japan	*Columbidae*
RVA/RefSeq/USA/2008	NC011500 - NC011510	USA	Unlisted
RVA/Human-wt/China/SD20200276/2020/G3P[19]	PP869338 - PP869348	China	*Homo sapiens*
RVA/Bovine/Iran/NZK-Fars-1/2009/G10P[11]	PP457634 - PP457644	Iran	*Bovine*
RVA/Human/VietNam/RVN15-0138/2024/G8P[8]	LC784433 - LC784443	Viet Nam	*Homo sapiens*
RVA/Human-wt/Guatemala/3000433433/2015/G1P[4]	ON736902 - ON736912	Guatemala	*Homo sapiens*
RVA/Human-wt/Paraguay/3000503796/2016/G3P[8]	ON736924 - ON736934	Paraguay	*Homo sapiens*

Table S4: (cont'd)

Rotavirus A Strain	Accession numbers	Location	Host
RVA/Human-wt/USA/3000765402/2017/G12P[8]	ON737056 - ON737066	USA: Los Angeles	*Homo sapiens*
RVA/Cat-wt/Thailand/CU25045/2020/G3P[3]	OR501082 - OR501092	Thailand	*Felis catus*
RVA/Human-wt/Italy/PA222/2021/G1P[8]	OQ815902 - OQ815912	Italy	*Homo sapiens*
RVA/Human-wt/Japan/HC12016/2012/G1P[8]	AB848004 - AB848014	Japan	*Homo sapiens*
RVA/Pig-wt/HRV/06-2017-Medj/2017/G3P[23]	OQ333046 - OQ333056	Croatia	*Sus scrofa domesticus*
RVA/Raccoon/USA/D2213150/2022/G3P[3]	OP947214 - OP947224	USA	*Procyon lotor*
RVA/CommonShrew-wt/Germany/KS11-0893/2010/G42P[58]	ON988153 - ON988162	Germany	*Sorex araneus*
RVA/JungleCrow-wt/Japan/JC-105/2019/G40P[56]	LC634422 - LC634432	Japan	*Corvus macrorhynchos*
RVA/Chicken/Germany/06V0661G1/2006/G19P[31]	MN365934 - MN365944	Germany	*Gallus gallus*
RVA/CommonGull-wt/Japan/Ho374/2013/G28P[39]	LC088218 - LC088228	Japan	*Larus canus*
RVA/Turkey-tc/Germany/03V0002E10/2003/G22P[35]	JX204822 - JX204832	Germany	*Meleagris gallopavo*

**NCBI reference sequences selected for viral consensus genome assembly using CZ ID platform.*

APPENDICES C

The following sequence is the vial assemble genome of pigeon circovirus (PiCV) obtained in this study. The full-length is 2035 bases, with 21 unknown nucleotides. A complete genome of *rep* gene, with 948 bases is assembled (the nucleotide sequence was coloured in blue; located at 48th to 995th nucleotide). A partial genome of *cap* gene, with 819 bases is assembled (the nucleotide sequence was coloured green; located at complementary 1167th to 1985th nucleotide). The conserved nonanucleotide motif, 5'-TAGTATTAC-3' was highlighted in yellow. Four forward repeat hexamers, 5'-GGAGCC-3' (two of which appear as 5′-CCTCGG-3′, the reverse complement of 5'-GGAGCC-3'), were highlighted in pink.

>PiCV/Malaysia/10-2024/2024/PQ570498
CCGCGACTTGGAGCCACGGAGCCACACATTGCGAGCGAAGCTGCAATGGAGGC
TCCGAGCAACCCACCCCCCCCAGGCCGCGTGTCCGCCGCCGCAGGCGGCGCCC
GGCGCGTAGCGCCCCGTCGCCCTCCCCGCGAGGCTGCAGCTAAGAGATGGTGC
TTCACCCTGAACAACCCGACCGAGGACGAGATNNNNNNNNNNNNNNNNNNNN
GTTGAGTGAGTTCCACTATGCTATAGTGGGGAAAGAGGTCGGGGAACAGGGTA
CTCCGCATCTGCAAGGCTTCGTGCACTTAAAGCAGAAGAAGCGACTACCGCAG
CTTAAGCAGCTTTTTAAGCGGGCGCATTGGGAGAAAGCTCGGGGCAGTGATGA
GGACAATGAGAAGTATTGCTCTAAAGAGGGTAATGTCCTACTTACTCTGGGCA
TTCCTGCGAAGGGAAACCGGAGCGACCTCAGCGGAGCTGTTGAGGCTGTGAAA
GCCGGAAGAGCAATGACCGAGGTCGCGCGAGAGTTCAGTGAAATCTACGTCAA
GTATGGGCGTGGTCTGCGCGACCTGAAGCTGCTGATTGGTCAGCAGCCACGTG
ACTTCAAAACGGAAGTCATCGTCATCACGGGCCCGCCTGGTTGCGGGAAGAGC
CGTTGGGCCCACGATTACCCCGGAAGTAAATTCTATAAGATGAAGGGGGAGTG
GTGGGACGGGTACGACCACCAGGATGTAGTCATCATCGATGACTTCTACGGCT
GGTTGCCCTTCTGTGAGCTGCTCCGTGTGACTGACCGGTACCCGCATAAGGTG
CCCGTGAAAGGGGCCTTTGTGGAGTTCACGTCTCGCGTGATCATAGTGACCAG
CAACAGTCCCCCCGATGCCTGGTACAGCGAGGAAAAGTGCTGTGTCCAGGCGC
TCTTCCGGCGGATCAACAAGTGGCTGGTCTGGAATCACGACAAGTTCGAAGAT
GCTCCAGATTGTATGAAAAAGTACCCCATCAACTATTAGCCCCCCACCCCCAT
GACACTAGTAAAGGGACCCAAGCCAGCCGAGCCGGCCGCGCCCGCGAGCGAAG
CGAGCGGCCGCGCGGCCTGGCGAGTCTGGCGGGTCCGGAGCGGAGCGACCGCG
AATGCGGGAGCGGAGCGACTACACTCGAAAATGAATTTGCAATAAAATGTTTTT
ATTCAGAATCCACAGCTGAGTCTGGGTCTAATGCATCTGCAAAACACTGGTTA
CAATCCCCGTTACAAATATGCATCAATTCCATGCCCTCAATGTTGGGGGTCGG
GGGGACATTGAGGGTCGTCCAGGCAAACTGTCTGAACTTTACATACAGTGTCA
CTTCACACTCATAATACATGTCGGTTGGCTGAGGTTGCAGGTAACTGAATGCA
AGCCCATAGTGATTAACCTTCTGTGGAAACCAGCTGTTTCCTGATGTCTGCAG
TGGTATCCACTGGTTTCTGCCTGAGAACCAGGTTGCTGCTGTCTGGTTTGCGG
TGGAGAGATCATTGATTGTGAGTTGGGGTCTTGGCCTTAGTAGCCGTTTAAAC
CCCTTTCTGAGATCCCATTTTCTGGCCCCATCAAAGTCCATGAGGGGGTCGTC
CCCCAGGTCTACCTGGCCTTGAAATGTTTTGAGCCTGGCATCATACATGGGCA
CTGTGTGCCCGAATCCTTTCCATGTTGTAATGTCAACTCCTAGTGGTCGCATC
TCCACCTTTGCTAACGCTATCTGATAGTCCTCAAAGGGGACTTTCAGTGTTGG
TGCGTTGAGGCCCACTGTGAGTACATCCGCTAGTTTGAATGTAAAGATTCCGG
TTCCAAATTTGAAATCATTGGTCGCTGCCGTCAGTGTGATCTTGTCCTTGCGA
CGTAGGCGGAAGTAGTAGATACGGCTACTCCTCCTGTGGCCACGTCTGTAGGG
GCGTGTCCTCCGTCTCCTGATCCGGCGTCGGCGAATGGGCGCCCTCTTACGGC

GGAATCTCCGCCTCCTCGCCATCTACGCAAACGTGACGTCACTTCCGTGGCTC
CAAGTCGCGGAATAGTATTAC

APPENDICES D

The following sequences are the viral assemble genome for rotavirus A with 11 segments, obtained in this study.

Segment NSP1:

>RVA/NSP1/Pigeon/Malaysia/Kpr/2024/G18P17

TGAGCAGTGGTGTTGGTGAGACGAGATGGCTTCAAACCAATTAAGATT
TGAGTATGCCATTGTTAAGTTTGCTAAATCATCTTTTACTTCATCTTT
AAAACCAATTCATTACACAAACTCAATGCAATGGACTAGTGATGATAA
ACTGAACAGACATTTGTGGAGAACATTTAACAGAACGATGATACATGA
TTTACCAAAAGGAAATTGTTGGACATGTGGAATATTCCATAATGTTTT
CGCTTGTGATTACTGTAATATAAACCATGTGTGTAGATCATGTAAAGA
ACATAGGATTGACTTGTGTCCATTTGTTAATGACACAGAGTGTAGATA
TTCACATGATCTCATTAGAATTGCAGATTGTACCGAATTGTATGATGA
CCTATTGTTGAAACATTTGCTACCGTTGTACAAGCAATATTACACGGC
ACTTAGTAAAATCTCAACAACAGCTGTTTGGAAAGAGTTGAAAGAGAA
GAGGAGAAGAGGCCAGCGCATGCATACTCTATCAACTATTGCCGTTGA
TTTTAATGATTGCTTCCTGCCAACAAATGTTATCGCATTTAAATCGAT
AGACCACAGGAAAATGACTTCTGAAAATAAAATATACATGAATTTATT
GTTTGGACACTACGAACCTCAAAGAAATAGAGAAGAAGGAATTAGTTA
TACTAACGTGAATTTGAAACAATTCAGGAAGACTTATGATGCAATAGT
ACAATTAAACTTACAATTGGCTATGCACAGAGGAAAAGCAGCAAGACC
TATGGTATCACTTGATATAACCAGAAACAGAGAATGTGTTCCATTCAT
TAACGATCAGGAATATATGTGTCAATTAAATGATAAGAAAGTTAATTA
CGCTTTATTGCACTATGATACATCTGACAAGTTTTTGTTATGCCGTCC
AATATTATCATGCTTGCAGCCAGAATCGTTGCTAAAAGAGATTTTCGC
ATCATGGTTGACAATAAATCCCAGAGTCGAACTGTCAAAATTACAAAA
GCAGCACTACAATTCCATATTCACCTTAGCACATACTCTGAAATGGCC
AAATAAGCACAGAAATTATATTGGATGGACTCATTTCTCATATTATGA
GAATTTAGTGCATGATGTGCTGGACAATTTGGAAGACATTAGGATGAT
TGCGGGAGCAGGAATACATTATGCGAAACACACATCGTTTGAAAACAA
CAATTGTGTTGCATGTAGAATGTACAACAAGTTTCTGGACGTTACAGT
AAAACAGAAAATAAATAAAATCTTAACAACGACATGTTGCTATCATGA
TTCGAGGTGTTGTGATCTTAACACTATTTTTACTATATCGCACAGAAG
AAATCACTTTGAACTAGAGTGGAGAACCATGTTTGATATAAGATCAAT
GGTTCTGCACTACGCTAGATTAACGAGTGAATTGCTTGACAATGATGG
CTTGCGGAGTGAATATGACATGATGCCAGAATGGAGTGACTGGCTACG
AAACGAATCTGATTATCTAGTGGCTACTAATGAAGTTGAGAAATTAGA
CGTCCTCCACAAAACGTGGTCGTTATCACAAGAAATGAGAAGAAAAAT
AATGGACAAATTCGAAGTAAATATTCATTACTATAAAGTATTTGAAAT
TCTAGCAGATGATTTTGAAGTCATACTTTCAATATCTACTGATGAAGA
CTTGGAAATGGTAGAAAGACCTAATTCATATAGATGGCTAGCAACAAT
TTTACCAGTTGAGGAAGATGAAGATTAACTTATTAACTTATACCAATT
GTTATTATTATTAAACATTACATGAAGCCTCGTAACATCATTGCGATT
TTGGAGTAGTGCCCAAAATGGCA

Segment NSP2:

>RVA/NSP2/Pigeon/Malaysia/Kpr/2024/G18P17

ATGCTCTCAGCTAAAATTGATAAAATTAATTCATCCCAACCATATGAT
ACACTGGTTTATGGACTAGCGCCACCACCAATTTATAAAAAGCGATTC

AATGATGGAAATAATTCTAGAGGGATGAACTTTAATACTGATATGTAT
GATAAAGTAGCGGACTTACTTGTACAAATATTAAATGGAATTAAGATT
GGAAAAGATAAAGCTGCTGAAATAATGGCAGTTCCAATTTCAGTACGT
CATTTAGAGAACTTAATCCATAGGATTGAAAATAAAGATGACACACTT
TCAGCAGATCCGAATCTAATTACTAAATCAGTATTAATAGCAATGGGT
TTAATTAAAGATTGTGAATTGACGGCTACAGCTGAAGGAGGGGATATT
GTCTTCCAAAATCAGGGCTTCACAATGTGGAAACTTGACTACAAATCT
CATGCTTTAATGCCAATCACTGATCCAAACTTTATTGAGTATAAAATA
ACATTAAATCATACTGATCCTATTGACGATAAAATAGTTAAGGAACTA
GTTGCTGAATTGAGATGGCAATATAATAAATTTGCAGTGATTACACAT
GGCAAAGGGCATTATAGAGTTGTTAGATACTCAACTGTTGCTAACCAC
GCTGATAGAGTCTACTCAACATTTAAATCAATCCAAAAGAGAAATCCG
TCGTATAAATTTAATGAATTAGATACACGTGTCGTTTGGACAAACTGG
GCTGCATTTGTGAAATCTATGCTGAATGGGATGAAACTAGATGATTCA
AAGCGTCTGCTTTTTACAAAAATGAAACCTAATGAATCACCATTTAAG
GGAGTCACGACGGAAAGAAAACTTGATGAGGTGTCACTATTAGGTTAA
AGTACCGCATGGGGATGCGATAGCAGGAATTGAAAG

Segment NSP3:

>RVA/NSP3/Pigeon/Malaysia/Kpr/2024/G18P17

CACATCGAGGCATTGAGAACATTGCTGTTCAGCGCTGTGTTTGTAGGT
AAAACATCAGACCTGAGATGGAAACGACTGTACTTGCAGTTAAATCAA
TCCTTTCTGCTGCCTTCGATTGTGCCGTAGCAACAACGAAGTCTATAC
TTGATGAACAGCAAGTCACATACGATGATGCCGAGATTGAAGCAAAGA
TACGTTGTAAGATGAATTTGATTCTGGATGATTCTGGTGTACAAGCTA
ACATGTTAGGAAAAGCTGCCACGATAGATCAGGCACTTAGTGGGAAAA
TGGCGTCAATGAATCGAAATGCTAACTGGATGACTACTCAATACACAG
TTGCTAAATTGGATGAGGACGTTAATAGACTAAGGATGATGCTTTCTG
CTAAAAATATTGACCAAAAAATGCGCGTTCTGAACTCGTGCTTCAAAG
TACAACGCAGCCTAACTAAGTCATCGAGCATAATAACTTGTACTAAAT
TACTCAAAGAAAAACTTAAACGCGGAGAAGTGATCGAAGTAGAAGATC
CGGAAGATGCAAGGATGGATGTCGATGTCAAAGAAAACATTGATTGGA
AAGGAAAGTTTGAACGCACAAAGATGAAGTTCCAGGAAAAATACAACT
CATGGGTGGCGAAAGCACGTAAAGTAGCGGATTCGGTTAAGAACATGC
AATTAACTATTGCAAACCAACAAGCTATGATAAACTCATTTGAAGAAA
AGCTTATGCAAAAAGACATTGAATATAAAACGAGAATCGAATCTTTGA
TATCAGCATTGGAATGGAAATTCAACTCAAAGCAATTTGAGGATGAGA
TTAGACAAGATTATCTGCAGGAATTGGAAACAATGACAGTGATTGATG
TGAATGATGGAGTGGCGAACATTGAAGAGTTCATTTATAAGTTGGTTG
ATGATTTTTCAGATTTGATACTGCTTTTGAAAGGATTAACTAATAAAT
TCGGGCTCACTGTAACATTCGATTAAACTACATCATAACCTCAACAGC
ATAGAGTCTCAGTGCAGTTAAAAACTAACTTGATGTTAAAATCGTGAA
TAACGCACGAG

Segment NSP4:

>RVA/NSP4/Pigeon/Malaysia/Kpr/2024/G18P17

GTACAACTTAACTATGAATTATTTTGAGCATAACGTTATACTAATAAA
ATATTTTCCATTCCTAGCGTCCATATTAACTATTGCTTTTACAGCTTG
GAAAATGGGCAAATCAACTTTTAAAATAACAAAATCTGTCGCTGGGTC
TGGATTTAAAGTAGTGCGCGTTGCTATTATTACTACTTTCAATTGTAT
AATGAAGACATTTGGCTCAAAGACTGAAATAGTTTCGGATGACAGAAT

TGATGCGTTAGCTACTAGAATACTTACTGAGATTGATAGACAGGTAAA
AATTATTGAGCAGCTAACAAAACGAGAACTAGAACAAGTGAAACTTCT
AGCTGACATTTATGAGCTGCTAAAATTCAAAAAAGATGAAATCGATAT
GTCATTCGAAACGAACAAAAAGGCATATGAGCAATGGATTCAAGATCC
ATATAAACCAACTCGAGCAGTGTCGTTAGATTAATGGCCCATGTCTGC
GTTGTCTTTGGAGGCGTAGAATGACG

Segment NSP5:

>RVA/NSP5/Pigeon/Malaysia/Kpr/2024/G18P17

CTACCGTGATGTCTGCAATAACTATCGACTTTTCATCTCTTCCATCAT
TTTCAAAATTATCAGCAAATGAATCATCTAGTGAAACGTCAAATACTT
CAAGAAAATCTGTTAGTAGGAGTGAGCAGTATGTATCGTTGTCCGAAA
AAGCATTTAGCGAATACATGCTTAATAGGGATCCAGAAGACATTGGAC
CATCTGATTCAGCATCAAATGACTTACCCACTAAATTTGCTATTAAAT
CAAATGCAGTTAAATCAAATGCTAATGTTGGCGTGTCGTTGGATGCTA
GCTCAGCAGATCGTGAGACAGGAAGTAATGCAAAAGATGAAGTCGATT
TCTCATTCGCTAAAGGAATAAAAATGAAATCAGATGTTGCTACATCCT
TCATTATTGATACTTCAAACGTAAAATCTTCAACTTCAGATCCAGGCT
CATCAACCAAATCGGTACATTATACGGATGTGATTAAATTAGGATCAA
AATTAAGAAATAAATCGTATAAAAATAATCAGAAATGGCCTAAGATAG
AATACCAATCCGACGAAGAGGAATTTGTATTAAGTGATGAAGATGCCC
AATGTTGTAATTGTGTTTATAAAAGAAAATATTTTGAACTAAGAAAAC
GTATGAAACTAGTAGCAATTCGGTTAATTGAAGACATGTAAGTCGGTT
TATTGCAGACTCACGAGGGAGATCCCCAMW

**Ambiguous bases: M indicates A/C; W indicates A/T*

Segment VP1:

>RVA/VP1/Pigeon/Malaysia/Kpr/2024/G18P17

CGATGGGGACGTATAATACCTTACTCTCGGAATATTTAAGTTTTTTAT
ATACTTCAAATGATTCAATACAAATACCAATTTATTATTCTTCAAACA
GCGATTTAGAATCGAGATGCGTGGAATTTCATGAAAAAGCCGTTAATT
TATCCAAAAATAATGAGTCAATAAAGCATTTATATGAGGAATACAAAG
ATGTGATAGATAAAGCGACATTATTATCAGTATTATCATATTCATATG
GTAAGTATGGGAACGTTGAGTCTAAAATAAAGGAATATGCCACAGTCG
CTCCATTAATTGCAGAATTTATAAATGATTTAGACTATGAGAATAACA
AATTAACTTCTGAATTATTCAATGAAGAAAATTATACTGATAGTTTGA
TGGATCCAGCAATACTTACTTCACTCTCTTCAAATTTAAACGCTGCTA
TGTTTTGGTTCCATAACAATAAGTCGAAAACGAAAACAGATCAAGAAT
TTGCTAAATTATATAGAAGAAGAGAAAGTCTATTCAGAATAGTCGCGT
CAACTGTTAATAAATATGGTGTTCCAAGACATGATCAAAAGTATAGAT
ATACGTATGATGTAATGAAAGATAAACCTTATTATTTAGTAACTTGGG
CAAATTCATCAATTGAGATGTTAATGTCAGTACATGATCATAATGATT
ATTTAATTGCTGAAGAACTTATCATTAACTCATATTCGAATAGATCAA
CTTTGGCAAAAGTAGTATCATCACCAATGTCAATTTTAGTCGCTCTAC
TTGATATTAATGGAACATTTATCACAACAGAAGAACTTGAACTTGAGT
TCTCAAATAAATACGTGAAAGCCATTGTACCGCAGAAAACTCTAGATG
AGCTAAATGCCATGTTAATTGCTATGGAAAAAGCAAACTTATACAATA
TACCGAAATTGATAAGAAATTGGATAGATGCACCAACACTAAGTGGTT
TTAAATTAATGGCTAAAATATATTCTTTCTCATTTCACGTTGGATTTA
GAAAACAGAAAATGATAGATGCAGCACTAGATCAGTTAAGTACAGAAT
ATTCAGATGATGTGGACGATGAGATGTATCAAGAATATTCAATGCTTA

TTCGGAATGAAGTGGTCAAGATGCTACAAGAGTCAGTTATACATGATG
ATCATTTATTAGTTGATTCAGAATTAGCTGGCTTATTATCAATGTCAT
CAGCATCAAATGGCGAATCCAGACAATTAAAGTTTGGAAAGAAAACTA
TTTTTTCAACAAAGAAAAACATGCACGTGATGGATGACATGTACAATG
GAAAATATAACCCTGAATTGGTACCTAAAGTTGACCAAACGCGTCCAA
TACCATTAGGTAGAAGAGATGTACCAGGTAGGAGAACCAGGATAATAT
TCATTTTACCATATGAATATTTTTTAGCTCAGCACGCAGTGGTGGAGA
AAATGTTACAATATGCTAAACATACGAGAGAATATGCGGAGTTCTATT
CACAATCAAATCAACTGTTGTCATATGGTGACGTAACGCGATTCTTGT
CGGATGACGCACTTGTATTATATACTGACGTATCACAGTGGGATTCAT
CGCAGCATAATACTGAACCTTTCAGAAAAGGTATAATAGCTGGATTAG
ATAGATTAATGACAATGACGAACAACAGGAAAGTTAAAGAAACACTCG
AAAAGTACAAACAAACACAAATAAATTTAATGGATTCATATGTTCAAA
TTCCAGATGGTAATAAAATTCTGAAGATTCAATATGGAGCAGTGGCAT
CAGGTGAAAAGCAGACTAAAGCTGCAAACTCAATAGCCAACTTGGCTT
TAATTAAAACCGTATTGTCTAGACTTGCAAATAAATATGCATTTAAAA
CTAAGATAATAAGAGTTGATGGAGATGATAATTACGCAGTGTTACAAT
TCAATTCGAAAGTAACACCAGAGTTAGTGATACAAGTGTCAGATTTTA
TAAGAGATACATACTCAAGAATGAACGCCAAAGTGAAAGCGTTAGTGT
CAACTGTGGGTATAGAAATTGCTAAAAGATATATCGCTGGAGGAAAGA
TTTTTTTCAGAGCTGGAATTAATCTACTTAATAACGAAAAGAGAGGTC
AAAATACGCAATGGGATCAAGCAGCCGTTCTTTATGCTAACTACATTG
TTAATAAGTTAAGAGGATTTAGTACTGATAGAGAATTTATCTTGACTA
AGATAATGCAAATGACTTCAGTTGCAATAACTGGATCTTTGCGACTGT
TTCCTTCAGAAAGAGTATTGACTACGAACTCAACATTTAAAGTATTCG
ATTCAACTGATTTCATAATCGAATACGGAACTACTGACGACGATGTGT
ACTTGCAGCGAGCTTTCATGTCATTATCGTCGCAATCATCTTCAATAG
CCGATGAAATCGCTTCATCTCAAGTGTTCAAAAATTATGTGAATAGAG
TGTCAAATGATCTATTAAAATTTAATAACTCAATAGTTGCTAAGGGTA
TTGCACAGACCGAGAAAGCCAAGCTAAATAGTTACGCGCCAATTGCAT
TAGAAAAGAGAAGAGCGCAACTATCATCATTATTAACAATGCTACAAA
AACCAGTTAGATTTAAATCTTCAAAAATTACTATAAACGACATTCTTT
CTGACATTAAACCATACTTTAAACAAGATGAATCGAATTTAGATCCAA
TATATCCAGAATTCATGCCGACTCTACCATATAATGTTCAATATGTAT
TGCGATGCATTGGCTCTAGAACGTATCAAATTGAAGATGACGGTTCAA
AATCAACTATATCGAAACTGATTAGAAAGTATTCTGTATACAAACCTT
CAGTGGAAGAGCTATATAAAGTTATTTCTCTCCAAGAAAGAGATATTA
AATTGTACCTAATGTCACTCGGTGTACCAGATATAGACTCGTCTGCTT
ATATAGAATCACGTGTTTATAACCAAGATAAATATAGGATATTAGAGT
CATATATCTATAACTTATTATCGATAAATTATGGATGTTACCAATTAT
TAGATTTTAACTCAAAAGACTTAGAATCGTTGATCAGAATACCATTTA
AAGGTAAAATACCATCAGTGACATTCATACTACATATTTATGCGAAAT
TACATATTATAAACACAGCGATTAAAACAGGGAAATGGATAACATTAT
TTTGTGATTTTCCAAAATCTGAAATGATTAAGCTGTGGAAGAAGATGT
GGAGTATAACTTCTTTAAGATCACCATACTCAAACGCTAATTTCTTCC
AAGATTAAGGTGCTAAGATGTG

Segment VP2:

>RVA/VP2/Pigeon/Malaysia/Kpr/2024/G18P17

TGGCCTACAGGAATAAGCGCAGTCAAGGAAATAAGAAGCAAGATACTA
AAACTGAGCCTGAGAATGATCAGCAAAATTCTAAAGAAATTAAGACTT

CACTTAAAGTAGATAAAACTAAGTCAGAATTGAAAGAAAAAGTATTTG
ATAGGAAAGACACAGTAATAACGGATGATCCACAACAGGATAAGAAGG
ATGTACTGCAAAATAACAACCTTAAACTAGATGAACAAGTGGCAAATT
CAAAAAAAGAGGATTCAAAACAATTAGTAGAAGTATTAAAGACAAAAA
AGGAGCATGAGAAGGAGGTACAATATGAAATATTACAGAAAACGATTC
CATCATTTCAATCTAACGAGACGGTGTTAAAGAAATTAATAGATATTA
AACCAGATCCAGCCAAAAAATCAGAAAAATTATTCAGGTTATTTGAGC
CAAAGCAACTTCCAATATATCGAGCGAGTGGTGAACGCGAATTGAGGA
ATAGATGGTATTGGAAATTGAAAAAAGATGACCTACCAGAAGGAGATT
ATGATGTCAGAGAGTACTTTTTACACCTTTACTCGCAAGTACTCGAAT
ACATGCCAGACTACATGCTTCTGAAAGATATTGCAGTGGAGAATAAGA
ATTCGCGTGATGCTGGAAAAGTGGTTGATAGTGAAACAGCCCAGATCT
GTGACGATATATTCCAGGATGAGGAAACTGAGGGCGTAGTTAGAAGAT
TCATCGCTGATATGAGACAAAGAGTAAATGCAGAGCGCAACACAGTAG
ACTATCCGGCTATACTTCACCCAATAGATTACGAATTTAACCAATACT
TTTTAACACACCAAATTATAGAACCACTAACCAATGAAGTAATTTATA
ATTATATACCAGAGAGGCTGAGAAATGATCCAAATTATATTTTGAACA
TGGATGTTAACTTACCACCGACCGCTAGATACATAAGACCAGTCCTGC
TTCAGGATAGACTTAACTTGCATGATAACTTTGAGTCAATTTGGGATG
CTCTCACTAGAGCTAACTACGTTCTAGCCAGATCAGTTGTGCCGGATT
TGAAGGAACTAGTGTCAACAGAAGCGCAAATTCAGAAAATGTCACAGG
ATCTGCAGTTAGAAGCGTTGACGANNNNNNNNNAAACACAGTTCCTGA
CAGGTATTAATTCCCAAGCGGCGAATGATGCGTTCAAGACGATTATAG
CATGTATGCTTTCTCAGCGGACCATATCACTAAAGTTTGTGACATCTA
ACTATATGTCATTAATTTCAAGTATGTGGTTGATGTCCATAGTACCTA
CAACTATGTTCATACGAGAGTCATTAGTAGCATGTCAGCTAGCAGTCA
TAAATACAATCATTTACCCTGCCTTTGGATTGCAAAAAATGCACTATA
TTAATGGTGATCAACGCACTCCATTTATGATTGCAGAACAGCAGATAA
ATAACTTTCAAGTACAAAATTGGCTACACTTTGTCAATCACAATACGT
TTAACCAAGTCGTCATTGACGGAGTTATTAATCAGACGCTGAACGATC
AAATAAGGCGTGGTTTAATCATAAATCAGCTTATGGAGGCGCTGAATG
TACTGGCTCGACAGAACTTTCAAGCATATCCAATAGATTACCGTAGAT
CAGTGCAACGAGGAATATTGCTACTTGCCAACAGAGTAGGGCAGCTTG
TGGACTTAACCCGCCTTATGTGTTATAATTATGAGACGCTGATGTCAT
GTATTACTATGAATATGCAAAATGTCCAGACGCTGACCACAGAAGAGT
TACAATTGACTTCTGTTACGTCGCTCTGTATGCTGATTGGAAATACTA
CTGTCATTCCAGAGCCACGAACTCTATTTCATTACTATCAGACCAATG
TGAACTTTCATAACAATTACAACGAGAGAATAAATGATGCCGTCGCAA
TCATTGCTGCAGCGAATCATCTAGATTTGTATCGTAAGAAAATGAAGT
CAATAGTTGAAGATTTCTTGAAAAAATTGCATATATTTGACGTAGCAA
AAGTACCTGATGATCAGATGTACAGACTACGTGACAGACTCAGACGTC
TCCCAGTGGAAAGAAGACGTGTTGATGTGTTCACTATAATACTTAATA
ACATGCAGCAAATAGAGAGAGCTTCTGACAAGATTGCACAAGGAGTAA
TTATTGCGTATAGAGAGATGCGCTTAGACTATGATGAGCTGTATGGAT
TCGTTAACTTAGCACGAGACGTCAATGGTTATCAACAGATTAACTTAG
AAGAACTAATGCGATCTGGAGACTATAATCAGATAACGAACATTTTGT
TAAATAATCAGCCAGTAGCATTAGTTGGTGCCATTCCATTCATAACCG
ACTCAGCTGTAACGTCATTAATAGCGAAACTGGATGCTACAGTCTTTG
CGCAAATTGTCAAGCAGAGGAAGATGGACACTATTAAACCAATTTTAT
TTAAGATTAACTCTGACTCAAATGATTTCTATCTAGTCGTTAACTACA
ACTGGATACCAACGTCAACGACAAAAGTTTTTAAGCAAGTGCCGCAGG

CTTTTGACTTCCGTGCAGCAATGCATGTACTGTCATCAAATCTGACTT
TTACCATGTTTCACGAATTGTTGCCGTTTGTAAACGCTGACACAGTTG
AACCAATTAATGCTGTCGCATTTGACAACGTTAGAATTATGCAAGAAC
TGTAAGCCATTACCGTAAATRGTG
**Ambiguous base: R indicates A/G*

Segment VP3:
>RVA/VP3/Pigeon/Malaysia/Kpr/2024/G18P17
CACAAGTAGTGCGTCTTACTCTTAGTGGTGTAGGCATGAAAGTGATAG
CTTTAAGACGCGACCTGGTCTCATCATATGCAGATACTCAAGTCTATG
AACATGACGTTAACAAAGATTATTACGAAAACGCATATTTAATCTCAA
ATATAACGACACATAATGTTTTATATGTTAATTACTCGATACAAGTGA
TTGAGATACTTAATAAATCAGGAATTGCATCACTTGTAGCTGTCAACA
AGGATGAGCTAGAAATTCTAATAAAATCTAATTTTACATATGATTACT
ACAGGAATATTGTTTACTTGCATGATTATTCATATTATAACCTTAATG
AAATACGTACTGATCAATATTGGCTGACAACTACTAATATTGAAGAAT
ACATTCTGCCTGGTTGGAAATTGACATATGTTGGCCAACTAGGAATTA
AGACCAGAGGTCATTACACATACAGCTTTATATGCCAGAATACTGCTA
CTGATGATGATATTATATATGATTACATTTATTCTAACGAAGTTGACT
TTTTGCCTTTTTTGCTTCAAGCATTAAATGAAAGATTGACCACAGCGT
TAAACTTTCACAGACTATCAAACAGAATATTTAGAGAATATTTGTATT
CAAAGGTGCCAAAAGACACTATTAACATTGGACCAAGAAACGAATCCA
TGTTCACACTGCTTCGCTATCCATACATAATGAATTACGCAGCTAATG
AATTTAAAGTATCTGACTTGATAAGACTTACTCAAGAGAAGTGGGTTG
GAGCCGAGAATTCACAATTTGATATTGGACAGTTTAAAAATATGTGTA
ATGTGTTATCAACTATTTATTACTATTATAATAAGTATCATGCATACC
CTCGAATTTATATGCTTGGTTCAGCGCCGTCGTATTGGCTATATGACT
TATTACAAGTACGTGATTTTGACATTGAAACGTGGGATCCATTGGACA
CACCATTTTCAAAACGGCATCACAAAGCCATGTTTACGATATCTGATA
CAGAGAAACTAAGTGATAATTCAATCCTTTACATTGACATAAGATTAG
ATAGAAATGGCGTTGATTGGAGAGGATGGAGGCAACGTGTAGAAGATG
AAACAAAAGTTAACTTAGAAATAATGTATAAATACCTACAGCGCGGAA
AAAATAGAATCTGTTGTTGTAAGATCACTGCTATGGATATTGAACTCC
CAGTCACATCTATACTACTACATTTTCCTACGACGAAGATACAGTCAG
AATGTTATGTTTTATGTACGCAAGAGATGTTGCAAGATAAGAAAAGAT
TTATACCGAAAGGAGCCTTTTATTCATTTATTAATAATACTAAAACTG
ATAACGTTTTTATTAGTCCAGTTTATAAAGTGAAACCAACAAATAAGT
TTGTTGTTGCGCTATATTCGCTTTCAAATGAGAGTAATGATAGAGATA
AAGTAATAGATTTTACGCAAAAGCAAAAGAGAGGCATTATCACATTAA
GGATGAATAATACTTTTAACTATGAGTACAGATTACAGTTTAAATCAA
CGTATGATTATTTATATCTGCCATCTGAAGTATCACGTGAAGGGACTA
TAGTTACATCATATGATGGATATATAGCAACGCATAATCTTTCTTTAT
CATTAGAAAGTAAAGCTACAGGGAATAATCACTTATTTATTTCATTTT
CTGACACGAATTATAACCAAATTGATTCATATTGTAACCATATGGGTA
TATCAAGAAGATCACATTCAGTCCGGTTTTCAGAAGCGGCTACAACTC
TATCTGGTTACATGTTTAGAGACATAACTAATGGAAAATTTAATTTAA
TTAATACAAATATAGAAAACGCAGTGTCCGGACACGTTTATAATGCAT
TAATATATTTTAGATATAATTATAGGTTTGATTTATTGAGATGGATAA
ATTTACATGCCAAGAATGAAGTCGTGATACAAGGGGGTAAATATTATG
AGCACGCCCCACCAGAATTGTTATATGCATGCCAATCAGCATTAGTAT

TTGCAAAATTGCAGAACGATTTAACGCTAATAGAATATGTAAACAGCG
TAAACCGCTATATCATAGCTAAATATGATATCAAATATGCTGATGATC
CAAATTACTATATACATATCGATTTTGTTGATTTACCTTTTAAGTACA
CTGTATCTGACCCACATTTGACTGGAGGATTGTTGTCATTAAATGACT
TTGACATTCAAAATGCAATAACGATATTAAGATCAGTAAGGGAAGATT
TGATAGCATTAAATTTATTAACTCAATACACATATATGTTAACTGATT
CAATTTATGTAGCGAATGTTGACGGTTACTTACAATTCTACTATAAGT
TATACATGAAATTTTATCGTAAGCAAATAGTATTCGGGCAATCAAGGA
TGTTCATACCTCATATAACCTTAGCTAAAGAGAAAAAGAAATCTGTGC
GTATAGATGCTACTAAACTCGTGATTAAATCAATTAAATTACGAAAAA
TTAAATCAGACGTAGAGTATTACATATAGATGGGATAGGATAAAAACC
TAACAGGTTTGTCGCAGATGTG

Segment VP4:

>RVA/VP4/Pigeon/Malaysia/Kpr/2024/G18P17

CGGGACCATTTGCGCAGACGGGTTATGCCCCAGTCGAGTGGACACATG
GTGACATCACAACAGATGTAACTGTACAACAAACTTTGGATGGACCAT
ATGCGCCATCTTCTGTGATGATTCAACCACAATATTGGGTACTAATGA
ACCCAGAGATTGCAGGTGTGATTGTTGAAGCAGATGCAACGAATAAGA
AATATGCTTGTGTAATGTTAGCGCCTAACACAGAAGAAGGTGACAAGC
AGTATGTTATACTTGGACACAAAATTACGATAAATTTGGGAAATACTG
ATCAAAATAGATATAAGTTCTTTGGTTTAGTTAGTAGGGATGGAGAAA
GGTATGTAAAGATACAAGAACTATTAACTCCTAATAGATTAAACGCTT
TCATGAAAGATCAAGGACGGTTGTATGTATATCACGGAACAATACCAA
ACATAACAACTGGATACTACACACTAGATGATATAGCGAACGTACAAA
CGAACGTTAAATGTAATTATTATATAATTCCAAAGGATCAAACTCAAC
GACTAGAAGGTTTCTTGAAAAATGGTCTGCCACCAATTCAAGAATCAA
GATACGTAATGCCAGTTGAGCGATTAGTACAAAACATATACCAAGCGA
AACCGAATGAAGATATAGTGATTTCAAAAACTTCACTTTGGAAAGAAA
TGCAATATAATAGAGATATTGTTATTAGATTTAAATTTGGAAATACCA
TAATTAAATCAGGTGGACTTGGATACAAATGGTCAGAAATATCATACA
AACCAATAAATTATGAATACACGTATGAACGAGATGGTGAAACAATAG
TTGCTCATACCACTTGTTCTGTTGCAGGTGTGAATGATTTTGGTTATA
TTTCTGGATCACTGCCAACTGATTTTGCTGTATCAAAATATGAAGTTT
TAAAAGAAAACTCGTACGTGTATATAGATTACTGGGATGACTCTCAGG
CATTTAAAAATATGGTATATGTACGATCACTAAGTGCAGAATTTAATG
CGATAAACTGTACTGGAGGTACGTATGATTTTCAACTTCCACTTGGAC
CATGGCCACGAATGCATGGTGGTAACGTAATACTTAATTCAGATGCGG
TAACTCTGTCAACCCAATATACTGACTTTGTGTCATTGAACTCATTAA
GATTTAGATTTAAACCTGCAGTTGGCGAACCATTCTTTGAAATAACAA
GAACAAGAGAGACCAGACTGTATGGTCTACCTGCAGCTAATCCCATGG
GAGAAAACGCATACTACGAAACAGCTGGAAGATTCTCACTAATATCTT
TAATACCATCAAATGACGACTATCAAACACCAATTCAAAACTCGACTA
CTGTCAGACAAGATTTAGAACAACAAATATCAGACTTACGGGACGAAT
TTAATCAACTGTCATCGGAAATAGCAATGTCTCAGCTAATTGACTTAG
CACTATTACCACTCGATATGTTCTCAATGTTTTCTGGAATTAAATCAA
CAATAGATGCAGTTAAATCAGTGACTACTTCAGTTATGAAGAAGATGA
AAACATCTACGTTGGCGAAATCTGTATCTACTATCACGGAAGAACTAT
CTGATGCTGCAACAAGTGTATCGCGAACATCGTCAATACGCTCAAATG
TTTCTGTGTGGAATGATTTAGTGGATGCAACTACACAAACCTCTGTAG
CAACAAATGATATTGCCACGCAAACATCACGTATTGCTTCGAAGTTAA

GAGTAAAAGAATTCGCAACGCAGACTGAAGGAGGACTTAGCTTTAATG
ACATTTCGGCTGCTGTATTAAAGACTAAAATGGATAAAATAGAGACTG
TACAACCAAAAATATTGCCAATAATCATTACTGAATCAATTGATAAGT
TCATTCCAACGCGTCAATATAGAATTATTGATAAAGATATTGCTTATG
AAATATCAAATAGTGGGAAATATTTTGCGTATAAGGTAGATACATTTG
AAGAAGTGATTTTTGATGTAGAAAAATTCGCTGATCTAGTAACTGATT
CACCAGTAATCTCAGCAATAATCGATTTTAAAACATTAAAGAACTTAA
ATGATAACTTCGGAATAACGAAAGAACAAGCGTATAACTTATTACGCT
CAGACCCAAGAGTGTTGAAAGATTTCATTAACCAAAATAATCCAATCA
TCAAAAATCGTATTGAACAATTAATATTACAATGTAGAATATAAATAG
AAGATGTCTATGAG

Segment VP6:

>RVA/VP6/Pigeon/Malaysia/Kpr/2024/G18P17

GAAGTCTCCATCATGGATGTGCTCTATTCACTTGCCAAGACACTAAAG
GATGCTAGAGCCAGGATAGTTGAAGGTACGCTTTACACTAACGTAGCC
GACATTATACAGCAAGTAAATCAGGTAATCAATTCAATTAATGGCTCA
ACCTTCCAAACTGGAGGGATAGGCAACTTGCCAATTAGAAATTGGACA
TTCGACTTTGGAACACTTGGAACGACCTTACTTAATTTGGACGCAAAT
TACGTTGAGAACGCAAGGACGACAATTGACTATTTTATTGATTTTGTT
GACTCTATTTGCGTCGATGAAATAGTACGTGAGTCTCAACGTAATGGA
ATAGCACCGCAATCTGATTCGCTAAGGCAACTTTCAAATGCCAAATAT
AAAAGAATAAACTATGATAACGAGTCAGAATACATAGAGAATTGGAAT
TTGCAAAATAGAAGACAGCGCACTGGCTATCTATTGCACAAACCAAAC
ATTCTTCCATACAACAATTCATTTACCTTAATTAGATCTCAACCAGCG
CATGATAACGTCTGTGGAACAATTTGGTTGAATAATGGATCTGAAATT
GAAATCGCTGGATTTGATTCTGAATGCGCATTAAACGCTCCAGGAAAT
ATTCAAGAATTTGAACATGTAGTACCAATGCGAAGAGTGTTAAACAAC
GCTACAATATCACTCTTGCCATATGCGCCAAGACTAACACAAAGAGCA
GTTATACCAACAGCTGACGGCTTGAATACGTGGCTATTTAATCCAATT
ATATTAAGACCAAATAACGTACAAGTAGAATTTCTGTTGAATGGACAG
GTAATAACTAATTATCAAGCTAGATATGGTACGTTGGCCGCCCGTAAT
TTCGATTCAATACGTATTTCGTTCCAACTTGTTAGGCCACCAAATATG
ACACCTGGAGTGGCAGCACTTTTTCCTCAAGCGGCACCATTTCCAAAT
CATGCAACAGTCGGTCTGACACTTAAAATTGAATCCGCTTCTTGTGAG
TCAGTTTTGTCAGATGCAAATGAGCCATATTTGTCAATTGTAACAGGC
TTGAGACAAGAATATGCAATTCCAGTAGGACCAGTGTTTCCAGCAGGT
ATGAATTGGACTGAGTTGCTTAATAATTATTCAGCATCTAGAGAAGAT
AACTTGCAACGTATATTTACAGCAGCATCCATTCGGAGCATGATTATT
AAGTAGAGATTAGGTGTAACAACCTATACCGAAGCTTAATAATTATGT
AGCTATACTGAAGGAAAATTCGTAGTGGACGTAAGCATTTGCGCTACC
CATGCAAGAATTW

**Ambiguous base: W indicates A/T*

Segment VP7:

>RVA/VP7/Pigeon/Malaysia/Kpr/2024/G18P17

TTTCTCACCGCGATTAGACTGTTTAAATGTATGGTACTGAATGTACTA
TCCTTCTAATTGAGATAATATTCTATCTTTTTTCGGCTATAGTTGTTT
ATGATATAATATATAAAATGGCTAATTCACCTATTTTTTGCATTGCTG
TTCTTGCTGTTGCTTTTGCCGCCTCTCCAAAATGTTTCGCTCAGAACT

ATGGAACTGATGCTCCTATCACTGGATCATTGGATGTAACAATACCTA
ATAAGACTAACGATCAAATAGGTTTAGTCTCTAGCTTATGTATTTATT
ATCCGAATGAAGTAGAGGGTGAGTTCAATGATAGCGAATGGAAAAGCA
CGGTGGCGCAACTACTACTGACAAAAGGTTGGCCAACCACATCAGTCT
ACTTAAATGGTTACTTAAATTTGCAATCTTTTTCAAATAATCCACAGT
TGAACTGCGATTACAATATAGTTTTAGTAAAATACGATCAGAATGTTG
ATCTAGATATATCCGAGTTAGCCGAACTGCTCCTGTATGAATGGCTGT
GCAACGAAATGGATGTCAGACTATATTATTATCAACAAACATCAGAAG
CTAATAAATGGATAGCAATGGGCAATGATTGCACGATTAAGGTTTGTC
CTCTAAATACTCAAACACTTGGAATCGGGTGTCGAACAACTGATGTAG
CGACATTCGAGCAGTTAACAACCGCTGAGAAGTTAGCCATAATAGATG
TTGTAGACGGAGTAAACCATAAAATCAATTATACAGTCGCATCGTGCA
CAATTAAAAATTGTATACGACTTAATCAACGGGAAAATGTGGCTATTA
TTCAAGTTGGAGGGCCAGAAATTATAGATATTTCAGAAGATCCAATGG
TTATACCAAAAGCGATCAGAGCGACACGTATTAATTGGAAAAAATGGT
GGCAAGTATTTTACACAGTAGTAGATTATATTAACGCTATAATTCAAG
CCATGTCAAAGAGATCACGATCGATTAACGCAAGTGCATACTTTTTGA
GGGTATAAACTCAATGTGAAG