




SHORT COMMUNICATION **OPEN ACCESS**

Avian Influenza Virus Surveillance Across New Zealand and Its Subantarctic Islands Detects H1N9 in Migratory Shorebirds, but Not 2.3.4.4b HPAI H5N1

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ABSTRACT

Highly pathogenic avian influenza (HPAI) virus subtype H5N1 has never been detected in New Zealand. The potential impact of this virus on New Zealand's wild birds would be catastrophic. To expand our knowledge of avian influenza viruses across New Zealand, we sampled wild aquatic birds from New Zealand, its outer islands and its subantarctic territories. Metatranscriptomic analysis of 700 individuals spanning 33 species revealed no detection of H5N1 during the annual 2023–2024 migration. A single detection of H1N9 in red knots (*Calidris canutus*) was noted. This study provides a baseline for expanding avian influenza virus monitoring in New Zealand.

Since highly pathogenic avian influenza (HPAI) A virus H5N1 first emerged in poultry in China in 1996, global trade of poultry

and recent spillovers to wild birds have resulted in the spread of HPAI H5N1 to every European, African and American country

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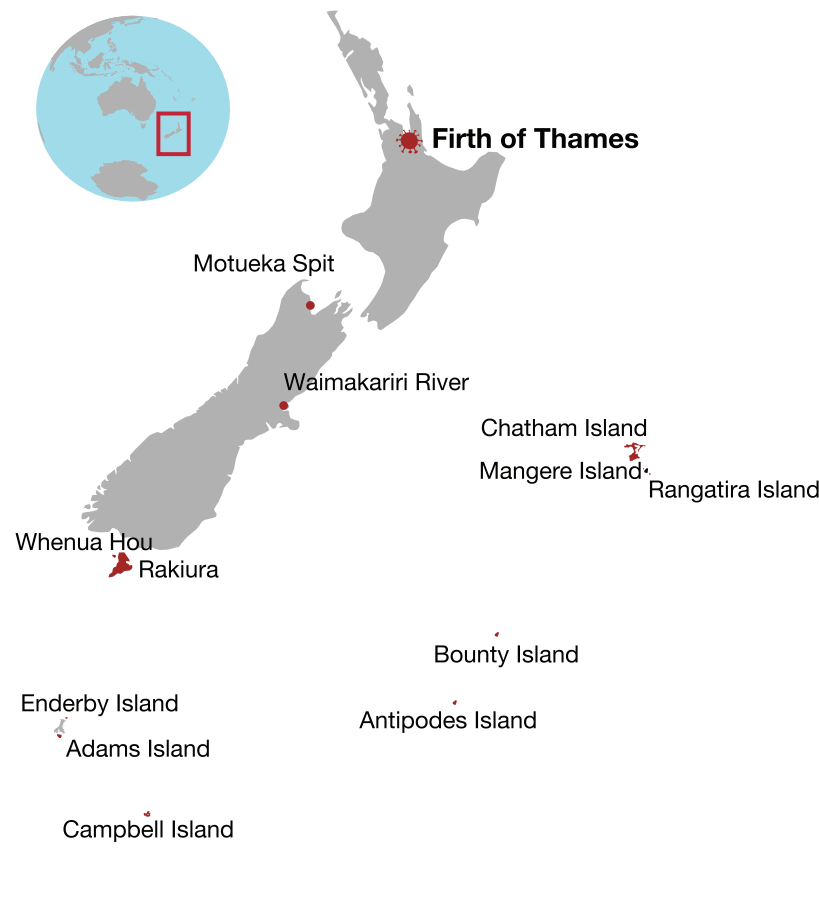
[1]. Since 2014, the subclade 2.3.4.4 has been the predominant lineage causing epizootic waves resulting in mass mortality events of wild birds and mammals, as well as poultry [1]. Oceania remains the only continent yet to detect HPAI H5N1 2.3.4.4.

Until recently, waterfowl have been the avian group believed responsible for long distance movement of avian influenza viruses (including HPAI H5N1 2.3.4.4) [2]. However, following the emergence of subclade 2.3.4.4b and associated epidemiological change of this virus, seabirds and shorebirds have also contributed to its long distance dispersal [3]. New Zealand's risk of HPAI has previously been considered low due to the absence of migratory waterfowl and its relative geographic isolation [4, 5]. However, the continued spread of HPAI to new geographic regions, including Antarctica, the increasing role that seabirds and shorebirds play in its dispersal and the rising number of susceptible host species have increased the risk of HPAI H5N1 being introduced to Oceania.

To date, New Zealand's avian influenza virus surveillance efforts have largely focused on waterfowl from mainland New Zealand [6] and have not encompassed New Zealand's offshore and subantarctic islands, which host a diverse range

of avian species including members of Charadriiformes, Sphenisciformes, Procellariiformes and Pelecaniformes [3]. Although H5N1 has never been detected in New Zealand, low pathogenic strains (LPAI) have frequently been detected through this surveillance over the past 20 years [6], and a local adaptation of LPAI subtype H7 to HPAI has occurred in chickens [7]. Given the global spread of HPAI H5N1 subclade 2.3.4.4b, including to the Antarctic peninsula [8, 9], the risk of incursion into New Zealand and its subantarctic islands has increased [3]. Due to this risk and the dynamic situation of HPAI H5N1, it is crucial that we expand our knowledge about avian influenza viruses harboured by aquatic birds in New Zealand.

Herein, we aimed to expand New Zealand's avian influenza virus monitoring by sampling seabirds and shorebirds from both mainland New Zealand and its offshore and subantarctic islands. Between November 2023 and March 2024 (i.e., spring to autumn), oropharyngeal and cloacal swabs were collected from 700 individuals across 33 avian species from 13 locations (Figure 1). Following similar protocols to those previously described [10], total RNA was extracted using ZymoBIOMICS MagBead RNA kit (Zymo Research). Equal volumes of extracted RNA were pooled into 207 libraries based on location, avian species and sample type. Pooled RNA was subject to total RNA



Location	Order	Species	No. of Individuals
Chatham Island	Charadriiformes	Chatham Island oystercatcher	10
	Procellariiformes	Fairy prion	13
Mangere Island	Procellariiformes	Grey-backed storm petrel	5
	Procellariiformes	Sooty shearwater	24
	Procellariiformes	White-faced storm petrel	8
Rangatira Island	Procellariiformes	Broad-billed prion	18
	Charadriiformes	Chatham Island snipe	15
	Procellariiformes	Sooty shearwater	6
	Procellariiformes	White-faced storm petrel	19
Whenua Hou	Procellariiformes	Common diving petrel	5
	Procellariiformes	Cook's petrel	7
	Procellariiformes	Mottled petrel	12
	Procellariiformes	Sooty shearwater	6
Adams Island	Procellariiformes	Whenua Hou diving petrel	12
	Anseriformes	Auckland Island teal	6
	Procellariiformes	Antipodean albatross	5
	Procellariiformes	Northern giant petrel	2
Antipodes Island	Procellariiformes	Sooty shearwater	2
	Charadriiformes	Subantarctic skua	3
	Procellariiformes	Antipodean albatross	11
	Procellariiformes	Northern giant petrel	12
Bounty Island	Charadriiformes	Subantarctic skua	4
	Procellariiformes	White-chinned petrel	9
Campbell Island	Sphenisciformes	Erect-crested penguin	25
	Procellariiformes	Salvin's albatross	24
	Charadriiformes	Antarctic tern	3
	Sphenisciformes	Eastern rockhopper penguin	11
	Sphenisciformes	Erect-crested penguin	1
	Sphenisciformes	Yellow-eyed penguin	112
	Procellariiformes	Light-mantled sooty albatross	4
Enderby Island	Procellariiformes	Northern giant petrel	15
	Charadriiformes	Red-billed gull	3
	Charadriiformes	Southern black-backed gull	1
	Procellariiformes	Southern royal albatross	31
	Procellariiformes	Storm petrel	3
Motueka Spit	Charadriiformes	Subantarctic skua	18
	Procellariiformes	Northern giant petrel	9
	Sphenisciformes	Yellow-eyed penguin	15
Firth of Thames	Sphenisciformes	Eastern rockhopper penguin	1
	Charadriiformes	Subantarctic skua	4
	Procellariiformes	Southern royal albatross	11
	Charadriiformes	Bar-tailed godwit	72
Waimakariri River	Charadriiformes	Banded dotterel	1
	Charadriiformes	Bar-tailed godwit	27
	Charadriiformes	Red knot	8
	Charadriiformes	South Island pied oystercatcher	6
Rakiura	Charadriiformes	Wrybill	22
	Columbiformes	Rock pigeon	6
Rakiura	Charadriiformes	Southern New Zealand dotterel	20

FIGURE 1 | Locations and species sampled during 2023–2024. Map (left) showing the 13 locations where birds were sampled during this study with the Firth of Thames in bold due to the detection of avian influenza virus in birds at this location. Table (right) listing the locations, taxonomic order, species (common name) and number of individual birds that were sampled. Both oral and cloacal swabs were taken from each individual, totalling 1400 samples.

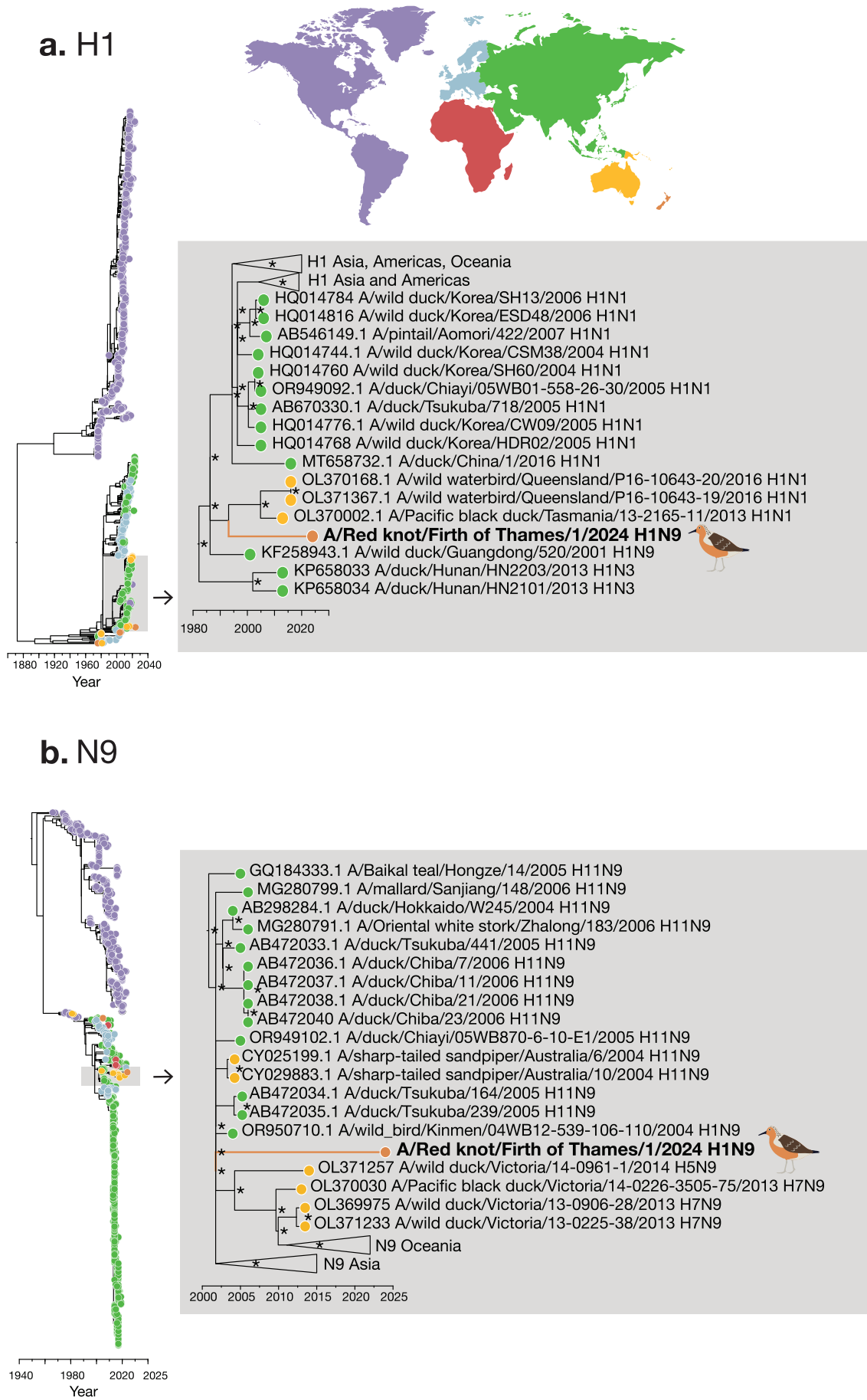


FIGURE 2 | Legend on next page.

FIGURE 2 | Time-calibrated maximum likelihood phylogenetic trees of H1 and N9 subtypes. Maximum likelihood time-scaled phylogenetic trees using IQ-Tree v1.6.12 [13] of (a) H1 and (b) N9. Each phylogenetic tree contains the closest 100 genetic relatives from a nucleotide BLAST search as well as all publicly available sequences within each segment subtype ($n=1332$ for H1 and $n=2010$ for N9) obtained from Bacterial and Viral Bioinformatics Resource Center (<https://www.bv-brc.org/searches/GenomeSearch>), aligned using MAFFT [14]. The enlarged images to the right of the phylogenetic trees shows the topological position of Influenza A/Red knot/Firth of Thames/1/2024(H1N9), with ultrafast bootstrapping [15] values of $> 70\%$, denoted by an asterisk. Tip colours represent the continents from which the sequences were sampled corresponding to the map above.

metatranscriptomic sequencing with ribosomal RNA depletion using Ribo-Zero-Plus. Paired-end 150 bp sequencing of the RNA libraries was performed on the Illumina NovaSeqX platform, achieving an average read depth of 62 (range: 41–145) million reads per library. Although highly sensitive real-time reverse transcription polymerase chain reaction (RRT-PCR) was not utilised here, metatranscriptomics has shown to be sensitive for virus detection and characterisation of all viruses infecting animals, including avian influenza viruses [11, 12].

While H5N1 was not detected in any of the 207 pooled libraries via metatranscriptomic sequencing, a near-complete viral genome comprising all eight segments (GenBank accessions: PQ358076-PQ358083) of H1N9 was identified in pooled RNA from cloacal samples from four red knots (*Calidris canutus*) sampled in March 2024 in the Firth of Thames, a site that is part of the East Asian-Australasian Flyway terminus and hosts thousands of migratory birds annually (Figure 2). A time-calibrated maximum likelihood phylogenetic analysis showed that the hemagglutinin (HA) segment fell into a broader Oceania clade with Australian viruses but diverged from a common ancestor of H1N1 viruses in Australia in the early 1990s. Similarly, the neuraminidase (NA) segment fell into an Oceania clade, related to sequences from both Australia and Asia (Figure 2). Red knots are a migratory species that arrive in New Zealand from mid-September. The detection of this virus in March 2024, combined with the phylogenetic position of HA and NA segments and the relatively large time gap between this virus and the last sampled common ancestors, suggests this LPAI lineage is not a new introduction to New Zealand. However, limited sampling of avian influenza viruses in New Zealand's migratory birds has hindered our ability to accurately resolve these lineages and determine their likely source. Increasing sampling efforts, particularly throughout the East Asian-Australasian Flyway, will help to clarify the evolutionary history and global connectedness of these viruses, enhancing our ability to better understand the transmission dynamics of avian influenza virus throughout this part of the world.

The identification of H1N9 in red knots at an internationally important coastal wetland has highlighted the need for enhanced avian influenza virus surveillance in migratory shorebirds. Approximately 30,000 red knots migrate to New Zealand annually from Siberia, travelling down the East Asian coast, making stopovers in Asia and Australia [16]. This migration poses a potential risk for the introduction of H5N1 into New Zealand. Similar to these findings, a recent investigation in Australia also found high prevalence and seroprevalence of LPAI in red knots as well as ruddy turnstones (*Arenaria interpres*), sharp-tailed sandpipers (*C. acuminata*) and red-necked stints (*C. ruficollis*), whereas very low antibody levels were detected in bar-tailed godwits (*Limosa lapponica*), great knots (*C.*

tenuirostris), curlew sandpipers (*C. ferruginea*) and sanderlings (*C. alba*) [17]. Although these species are from the same avian family, Scolopacidae, the differences in viral prevalence and seroprevalence suggest that there are interspecies differences that may alter avian influenza virus carriage, transmission and exposure potential [17]. Nevertheless, with the now wide geographical and host range of HPAI H5N1 subclade 2.3.4.4b, continued surveillance of a broad range of seabirds and shorebirds remains critical.

This work has established a framework for the broader surveillance of avian influenza viruses using state of the art genomics approaches, not only in mainland New Zealand but extending to birds located on New Zealand's offshore and subantarctic islands. Although HPAI was not identified in any of the birds sampled during last season's 2023–2024 migration to New Zealand, high-risk periods are imminent when migratory birds arrive back in New Zealand between September and November every year [5]. It is crucial to continue this enhanced surveillance to rapidly detect any viral incursions and build a longitudinal data set to better understand the dynamics of avian influenza virus evolution in New Zealand.

Author Contributions

Stephanie Waller J: conceptualization, investigation, writing – original draft, methodology, validation, visualization, writing – review and editing, formal analysis, project administration, data curation. **Janelle Wierenga R:** investigation, methodology, writing – review and editing. **Lia Heremia:** methodology. **Jessica Darnley A:** methodology. **Isa de Vries:** methodology. **Jeremy Dubrulle:** methodology. **Zoe Robinson:** methodology. **Allison Miller K:** writing – review and editing. **Chris Niebuhr N:** writing – review and editing, resources. **David Melville S:** methodology, writing – review and editing, investigation. **Rob Schuckard:** investigation, methodology, writing – review and editing, resources. **Phil Battley F:** investigation, methodology, writing – review and editing, resources. **Michelle Wille:** writing – review and editing. **Ben Alai:** methodology. **Rosalind Cole:** investigation, conceptualization, resources, project administration, writing – review and editing. **Jamie Cooper:** methodology. **Ursula Ellenberg:** methodology. **Graeme Elliott:** methodology. **James Faulkner:** methodology. **Johannes H. Fischer:** methodology. **Jim Fyfe:** methodology. **Lance Hay:** methodology. **David Houston:** methodology. **Bianca Keys C:** methodology. **Jenny Long:** methodology. **Robin Long:** methodology. **Thomas Mattern:** methodology. **Hollie McGovern:** methodology. **Lou McNutt:** methodology. **Peter Moore:** methodology. **Odin Neil:** methodology. **Jake Osborne:** methodology. **Anne-Sophie Pagé:** methodology. **Kevin Parker A:** methodology. **Mike Perry:** methodology. **Brodie Philp:** methodology. **Julia Reid:** methodology. **Kalinka Rexer-Huber:** methodology. **James Russell C:** methodology. **Rachael Sagar:** methodology. **Thor Ruru T:** methodology. **Theo Thompson:** methodology. **Leith Thomson:** methodology. **Joris Tinnemans:** methodology. **Lydia Uddstrom:** methodology. **Te Arawhetu Waipoua:** methodology. **Kath Walker:** methodology. **Edin Whitehead:** methodology. **Chrissy Wickes:**

methodology. **Melanie J. Young:** methodology. **Kate McInnes:** conceptualization, investigation, writing – review and editing, project administration, resources. **David Winter:** conceptualization, investigation, funding acquisition, writing – review and editing, supervision. **Jemma Geoghegan L:** conceptualization, investigation, funding acquisition, writing – original draft, methodology, writing – review and editing, project administration, supervision, resources.

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Ethics Statement

Animal ethics for sampling birds at the Firth of Thames and Motueka Sandspit were obtained from Massey University Animal Ethics Committee (MUAEC 22/52), and sampling was permitted via the Department of Conservation (Wildlife Act Authority 38111-FAU). All other birds were sampled by the Department of Conservation in accordance with their Wildlife Health Management Standard Operating Procedure.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Viral sequences have been submitted to GenBank under the accession numbers PQ358076- PQ358083.

Peer Review

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/irv.70099>.

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