Māui and Hector's dolphins: disease surveillance and tissue archive development

Annual Report 2024/25

10 June 2025

Wendi Roe, BVSc, BSc, PhD, Diplomate ACVP Professor of Marine Mammal and Veterinary Pathology Tāwharau Ora – School of Veterinary Science Massey University, Palmerston North

1. Context

This report forms part of a multi-year, integrated disease surveillance and tissue archiving programme for Māui and Hector's dolphins, initiated to support long-term conservation and health monitoring efforts. The 2024–2025 reporting period builds on foundational work undertaken since 2022, with a focus on enhancing molecular diagnostic capabilities, standardising tissue collection protocols, and establishing a robust archive of PCR-quality samples.

The programme is designed to generate baseline data on the prevalence and distribution of key pathogens— particularly *Toxoplasma gondii*, *Brucella* spp., and morbilliviruses—within the dolphin population. These pathogens are of significant concern due to their potential to cause both latent and fatal infections. The role that *T. gondii* and *Brucella* can have with reproductive success is particularly concerning. Morbillivirus, known in other cetacean species in other parts of the world, can play a significant role in immunosuppression that may increase vulnerability to other diseases. Their presence may reflect broader environmental and anthropogenic pressures.

By embedding this work within the larger Māui and Hector's dolphin necropsy and tissue sampling framework, the programme ensures continuity, comparability, and scalability of data across years. The outputs presented here contribute to a growing dataset that will inform future risk assessments, conservation strategies, and policy decisions aimed at safeguarding the health of New Zealand's endemic dolphin species.

2. Output summary

The Department of Conservation provided \$20,000 pa for 2024/25 to supply the following services:

- Collection, storage and archive management of PCR-quality tissues from dolphins submitted through the Hector's dolphin necropsy contract.
- DNA/RNA extraction from selected tissues to allow for routine surveillance for Toxoplasma, Brucella and Morbillivirus.
- Laboratory analysis to determine tissue/dolphin status for Toxoplasma, Brucella and

morbillivirus (prioritising *Toxoplasma*) as funds allow.

The following outputs have been achieved in the 2024/25 period:

- A standardised protocol has been developed for collection and storage of PCR-quality tissues from all suitably-preserved (i.e. non-decomposed) Hector's and Māui dolphins processed through the Marine Mammal necropsy contract.
- A frozen tissue archive has been established. This archive holds a standardised set of tissues for each of 40 individuals (1 Māui and 39 Hector's) necropsied between March 2022 and the current date, comprising approximately 400 tissue samples for dolphins with identification numbers from H300 H347. These tissues are stored so as to be suitable for downstream molecular testing (e.g. PCR) and microbiological culture. Frozen tissues are likely to remain stable for these purposes for at least a decade (if not used prior).
- DNA/RNA extraction for pathogen surveillance testing is conducted on selected specific tissues (e.g. lung, tongue, liver, brain for *Toxoplasma*), with extraction runs taking place in batches, once a suitable number of samples have accumulated.
- DNA extraction has been completed on a tissue subset for all 40 individuals.
- RNA extraction has been completed on a tissue subset for 29 individuals.
- Standardised molecular testing protocols have been developed and implemented for PCR-based detection of *Toxoplasma* and *Brucella*.
- A new protocol is being developed for detection of morbillivirus (see below).

3. Disease surveillance findings

Detailed results for individual pathogens are discussed below and show in Table 1, which summarises test results for 28 dolphins submitted between March 2022 and March 2024 (H300 – H332).

2.1 Toxoplasma

Toxoplasma can cause both fatal infections and latent infections in Hector's and Māui dolphins. Fatal infections are diagnosed by visualising the parasites in tissues using histology (examination of a full range of tissues under the microscope) and immunohistochemistry (microscopic examination using signal molecules that bind specifically to the parasite). Latent infections are diagnosed by detecting parasite DNA in dolphin tissues using PCR. The proportion of tested dolphins that are PCR-positive (latently infected) can give an indication of the broad level of exposure of the sample population. By regularly testing a standard set of tissues using a standardised protocol we will be able to identify trends in exposure and infection over time. The funding for this project will us to establish these protocols and to generate baseline data against which to compare future findings.

Over the past 12 months we have tested three molecular protocols to determine which is more sensitive, robust and economically viable. We have also completed a comparative tissue study to establish a baseline set of tissue types. We are now at the stage where we have identified an optimal protocol and are in the process of retesting all tissues with this standardised protocol. The results presented here are therefore provisional, since not all tissues have yet been tested with the optimal method.

To date in this study, 135 tissues from 28 dolphins collected between March 2022 and June 2025 have been tested for the presence of *Toxoplasma* DNA. Of these tissues, 19 were positive on PCR, belonging to 11 individual dolphins, giving us an overall prevalence of 11/28 (39%). Of the 11 PCR-positive dolphins,

one died of toxoplasmosis (H315) while the remaining 10 had latent infections, as established by histology.

Archived tissues for dolphins H333 to H347 will be tested over the next few months.

2.2 Brucella

Brucella infections have previously been diagnosed in both Hector's and Māui dolphins. As part of the current project 54 tissues from 27 dolphins H301 to H332 have been tested for *Brucella*, with none testing positive.

2.3 Morbillivirus

Morbillivirus testing has proven to be problematic. The PCR assay we have historically used detects a range of distemper and morbilliviruses. In the past 12 months we have had several dolphin tissues test positive, despite these animals having no indications of disease consistent with morbillivirus infection. This could represent latently infected/carrier animals, however genetic sequencing identified canine distemper virus (CDV), suggesting either that this was newly present in dolphins or that this was due to cross-contamination from the assay positive control. (Canine distemper has not previously been reported in cetacean species.) To further investigate this we asked MPI to test 4 of our 'suspect' dolphin tissues. They used 3 different assays on each of the 4 tissues, and found 11/12 tests to be clearly negative, with one being reported as having a 'weak, non-significant' signal. At this stage therefore we tentatively assume that these dolphins are <u>not</u> infected with morbillivirus. Our next step is to test and validate a new morbillivirus method, which we will undertake in the next 6 months or so.

| H no. | Path No. | Sex | COD category | Toxo L | Тохо В | Toxo T | Тохо Н | Toxo status | Brucella L | Brucella B |
|-------|----------------|--------|--------------------------|--------|--------|--------|--------|----------------|------------|---------------|
| H300 | 60555 | F | Maternal separation | Х | Х | neg | neg | neg | х | Х |
| H301 | 60475 | М | Open | neg | neg | neg | neg | neg | neg | neg |
| H302 | 60531 | М | Disease | neg | х | neg | neg | neg | neg | Х |
| H303 | 60839 | М | Open | neg | pos | pos | neg | pos | neg | neg |
| H304 | 60726 | F | Disease | neg | Х | neg | neg | neg | neg | Х |
| H305 | 61016 | F | Disease/Possible bycatch | noc | nod | nod | nod | noc | nod | nod |
| H307 | | | , | pos | neg | neg | neg | pos | neg | neg |
| H308 | 61391 61420 | M F | Open | neg | neg | neg | neg | neg | neg | neg |
| | 61469 | | Disease Known byggtab | neg | neg | neg | neg | neg | neg | neg |
| H309 | | F | Known bycatch | neg | neg | neg | neg | neg | neg | neg |
| H310 | 61470 | M | Known bycatch | neg | pos | neg | neg | pos | neg | neg |
| H311 | 61483 | F | Maternal separation | neg | pos | neg | neg | pos | neg | neg |
| H312 | 61547 | М | Trauma | neg | neg | neg | pos | pos | neg | neg |
| H313 | 61548 | М | Maternal separation | neg | neg | Х | neg | neg | neg | neg |
| H315* | 61824 | M | Disease | pos | pos | pos | pos | pos | neg | neg |
| H316 | 62074 | F | Known bycatch | neg | X | neg | neg | neg | neg | Х |
| H317 | 62201 | F | Known bycatch | neg | neg | neg | neg | neg | neg | neg |
| H319 | 62691 | М | Known bycatch | neg | neg | neg | pos | pos | neg | neg |
| H320 | 62907 | М | Shark bites | neg | pos | neg | pos | pos | neg | neg |
| H321 | 62906 | М | Known bycatch | pos | pos | neg | pos | pos | neg | neg |
| H322 | 62916 | F | Known bycatch | neg | pos | neg | neg | pos | neg | neg |
| H324 | 63194 | М | Known bycatch | neg | pos | pos | neg | pos | neg | neg |
| H326 | 63000 | М | Known bycatch | neg | neg | neg | neg | neg | neg | neg |
| H327 | 63084 | М | Disease | neg | neg | neg | neg | neg | neg | neg |
| H328 | 63085 | М | Trauma | neg | neg | neg | neg | neg | neg | neg |
| H329 | 63098 | М | Known bycatch | neg | neg | neg | neg | neg | neg | neg |
| H330 | 63086 | F | Known bycatch | neg | neg | neg | neg | neg | neg | neg |
| H331 | 63087 | F | Known bycatch | neg | neg | neg | neg | neg | neg | neg |
| H332 | 63130 | М | Known bycatch | neg | neg | neg | neg | neg | neg | neg |

Table 1. Molecular test results for tissues from 27 Hector's and 1 Māui dolphin.

Key:

H315* (bolded) is a Māui dolphin that died of disseminated toxoplasmosis.

Grey shading = negative (neg) on PCR; blue shading = positive (pos) on PCR; x = tissue not available.

'Toxo status' designated as positive when any of the 4 tissues tested positive.

H no. = Hector's dolphin identification number.

Path no. = Pathology identification number (School of Veterinary Science necropsy database).

F = female; M = male; COD = cause of death; Toxo = toxoplasma; L = lung; B = brain; T = tongue; H = heart.