

Re-establishing New Zealand's freedom from *Theileria equi* after the introduction of a single infected horse

In May 2020 *Theileria equi*, a cause of equine piroplasmiasis, was detected in a horse that had been imported into New Zealand 15 months previously. Consequently a biosecurity response was initiated. Extensive active surveillance confirmed that no transmission had occurred and that this animal was the only case present in New Zealand. A dossier was supplied to the World Organisation for Animal Health (OIE) and accepted in January 2021, re-establishing New Zealand's freedom from *T. equi*.

Theileria equi background

Theileria equi (formerly *Babesia equi*) is one of two intra-erythrocytic protozoan parasites responsible for the tick-borne disease, piroplasmiasis of equids (equine piroplasmiasis). This disease is characterised by fever, anaemia, icterus and in some cases death; clinical signs can also be mild and non-specific. The incubation period for disease following exposure is 12–19 days (Rothschild 2013). All equid species including horses, donkeys, mules and zebras are susceptible (Wise et al. 2014).

The time elapsing before a detectable serological response is variable, depending on the test used, with potentially up to 5 weeks elapsing before it can be detected by cELISA (Onyiche et al. 2020). Infection with *T. equi* results in a lifelong carrier state, with chronically infected individuals serving as reservoirs for transmission to naïve horses (Wise et al. 2014), although some treatment protocols have reportedly eliminated the organism (Ueti et al. 2012).

In countries where it is endemic, *T. equi* is primarily tick-borne, with several species of the genera *Amblyomma*, *Boophilus*, *Hyalomma* and *Rhipicephalus* recognised as competent vectors (Onyiche et al. 2020). Transplacental *T. equi* infection from carrier mares to foals has also been documented (Chhabra et al. 2012; Georges et al. 2011) and can result in abortion, stillbirth or neonatal

infection (Wise et al. 2014). Not all foals from infected mares are affected, and the prevalence of this type of transmission is unclear (Wise et al. 2014). Transmission can also occur iatrogenically e.g. through multiple use of needles, or through natural events that result in transfer of blood horizontally e.g. mechanically through skin damage and blood transfer during mating.

Case history

In April 2020, during routine export testing, a pregnant 4-year-old mare tested positive for antibodies to *T. equi* by cELISA and IFAT. Further testing on 22 May 2020 with a generic semi-nested conventional piroplasma PCR test, followed by gene sequencing, confirmed that the animal was infected with *T. equi*. Before this diagnosis New Zealand had never had a case of *T. equi*. The mare was clinically healthy and had no history of disease since being imported. Blood biochemistry and haematology confirmed that blood parameters were within normal limits.

The mare had previously lived in France before being imported in February 2019 via the UK. It met the requirements of New Zealand's import health standard, which included a negative cELISA test for *T. equi* during the 21 days before importation.

However, although the pre-import test was negative, an investigation undertaken by MPI in collaboration with the New Zealand Equine Health Association (NZEHA), determined that the mare had previously raced in both Germany and France, where *T. equi* is endemic, and had tested positive to *T. equi* by IFAT carried out on blood collected on 12 December 2018 prior to her pre-import testing. This result was not previously known to MPI. The investigation concluded that the mare was a chronic carrier of *T. equi*, and was already infected at the time of importation.

The mare was re-exported without treatment for *T. equi* on 26 June 2020, thus removing the only infected horse present in New Zealand. The importing

country was aware of the mare's health status. The mare foaled without complication, with the foal testing negative to *T. equi*.

Investigation

New Zealand has only one tick species that feeds on livestock, *Haemaphysalis longicornis*, but it is not a recognised competent vector of *T. equi*. Although transmission of infection was unlikely, a comprehensive investigation was undertaken to provide further assurance that there was no biosecurity risk to horses in New Zealand. This included examination of records, farmer interviews, site visits and blood sampling of all in-contact horses and horses that shared grazing with the imported mare (**Figure 1 and Table 1**).

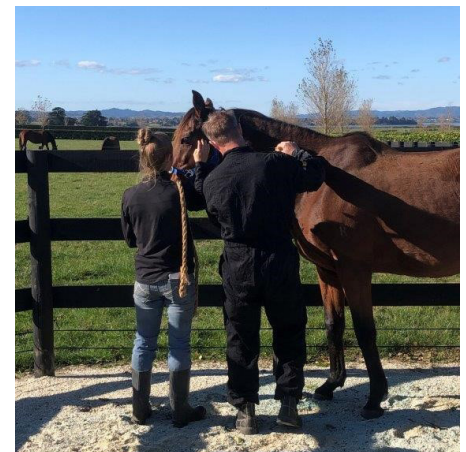


Figure 1: A Biosecurity New Zealand Incursion Investigator taking a blood sample from a horse during the early stages of the investigation

While in New Zealand, the mare was initially resident on a property at Karaka in South Auckland, arriving there on 16 February 2019 after 2 weeks in post-entry quarantine. She was then transferred to a sister property in Cambridge on 8 August 2019, where she lived until returning to the Karaka farm on 7 November 2019 (**Figure 2**). The two farms were part of the same Thoroughbred breeding stud. During her residence on the Cambridge property the mare was served by a stallion, which was subsequently re-exported to Europe (to a *T. equi*-endemic country) in

Table 1: Surveillance test results

Location	Number of horses	Number tested	Diagnostic test	Results
Cambridge property	204	5†	cELISA & PCR	Negative
		199	cELISA	Negative
Karaka property	143	13†	cELISA & PCR	Negative
		129	cELISA	Negative
		1#	cELISA, PCR, IFAT, gene sequencing	Positive
Movements traced from Cambridge and Karaka properties	180§	7†	cELISA & PCR	Negative
		11‡	cELISA * IFAT	Negative
		162	cELISA	Negative
Total	527	527		

† Horses with a history of direct contact (shared the same paddock) or with potential for iatrogenic spread from the infected mare, were tested with both cELISA & PCR

The single infected mare

§An additional 26 horses had been exported from New Zealand prior to detection, and one horse died prior to the sampling period (euthanased owing to severe laminitis)

‡ Eleven horses had been tested to meet new Australian import requirements initiated after the detection of the infected mare

September 2019. Following the mare's positive *T. equi* tests she was moved to an isolation area on 29 May 2020, from where she was subsequently exported. No clinical signs consistent with piroplasmosis were reported in the infected mare or any other horses from either property during the time that the mare was in New Zealand.

In conjunction with the NZEHA, all horses of all ages that had been present on the Cambridge and Karaka properties since the arrival of the infected mare, and those that had subsequently been moved to other locations in New Zealand, were traced and tested. One horse had died prior to the sampling period from an unrelated cause.

In addition, the horses that had shared the same flight into New Zealand as the mare were tested. The investigation identified that any horses exposed to risk of iatrogenic transmission had already been tested and were negative.

In total 527 horses were sampled and tested between 21 May and 21 August 2020. Of these, 204 were on the Cambridge property, 143 were at Karaka (including the infected mare), and 180 had been moved to other locations within New Zealand. An additional 26 horses had been exported and were subsequently traced by the importing country and tested (except the stallion that had served the infected mare, as it had

returned to a *T. equi*-endemic country). All tests undertaken returned negative results for *T. equi*.

Further activities

Export testing

As a consequence of this detection and the change in New Zealand's *T. equi* disease status, export partner countries amended their certification requirements for horse imports from New Zealand to include testing for *T. equi* using both cELISA and IFAT. All export testing returned negative results except with the single infected mare when she was exported. From 1 June to 30 September 2020 a total of 972 horses were tested, all with negative results.

General surveillance

New Zealand operates a comprehensive animal general surveillance programme, which is an essential part of our national post-border biosecurity system (Tana 2014). As part of this system, MPI maintains contracts with the three commercial veterinary diagnostic laboratories in New Zealand to provide services including (but not limited to):

- diagnostic information for surveillance;
- screening of submission forms for key words that could indicate exotic disease;
- notifying within strict timeframes upon suspicion of an exotic or notifiable organism; and
- maintaining minimum operational and technical quality assurance requirements.

Audits are regularly conducted by MPI to ensure compliance with contractual requirements. These laboratories receive samples taken by veterinary practitioners for routine sick domestic animal and wildlife disease testing, and provide MPI with monthly reports. This data is used to monitor and analyse trends in disease occurrence that might indicate the presence of an exotic equine disease.

During the 21-month period from 1 January 2019 to 30 September 2020 a total of 10,487 equine submissions were received, and diagnostics

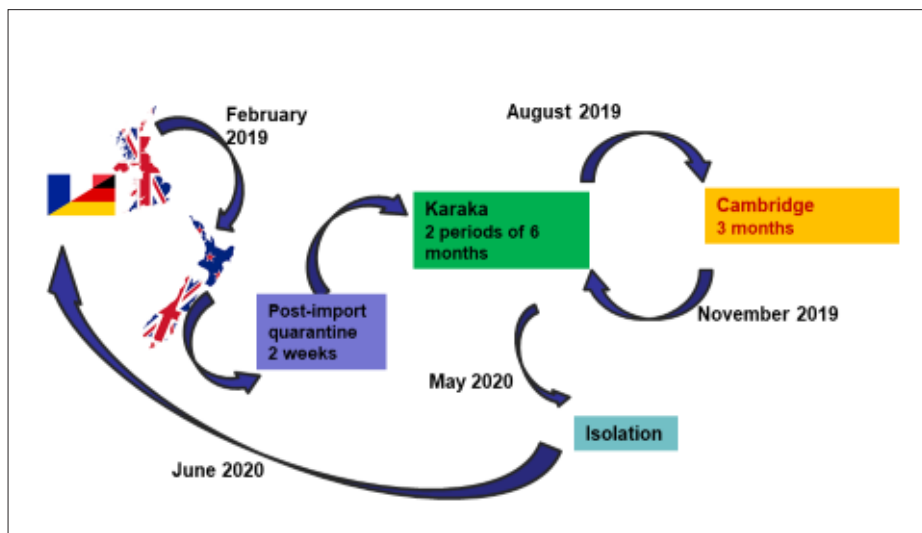


Figure 2: Schematic diagram summarising the movements of the mare infected with *Theileria equi* over the period from when she was imported until she was re-exported

undertaken, by the commercial veterinary laboratories under the supervision of veterinary pathologists. Haematological examination was conducted on 4,617 of these submissions. Further, MPI enhanced awareness of *T. equi* within the general surveillance system by asking the veterinary diagnostic laboratories to tell their staff and ensure that all equine blood smears were thoroughly assessed. *T. equi* was not detected in any submissions.

Import health standard

After the infected imported horse was identified, an amendment was made to New Zealand's existing mitigation measures for *T. equi* in imported live horses. Horses imported from countries that are not free of piroplasmiasis are now required to have both negative IFAT and cELISA test results prior to import, whereas previously only one test was required. This measure was implemented to reduce the risk of importing another carrier animal.

In addition, data on all horses imported into New Zealand since January 2018 that originated from countries not considered free from *T. equi*, were collected to determine which tests had been undertaken prior to importation. The NZEHA facilitated the prompt collection of this data on behalf of MPI. It was confirmed that all horses imported over that period (other than the infected mare) had been tested with both the cELISA and the IFAT prior to importation, with negative results on both tests.

The investigation and supplementary activities confirmed the absence of any disease transmission from the infected mare and identified her as being the primary and only case in New Zealand.

Re-establishment of freedom

The epidemiological investigation and subsequent surveillance activities determined that the mare was infected before being imported, she was the index case, and no other transmission event had occurred while she had

been in New Zealand. On 8 October 2020, in accordance with the relevant provisions of the OIE Terrestrial Animal Health Code (2019 edition), New Zealand declared it had regained its status as a country free from infection with *T. equi*.

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