We know from BVD bulk milk testing that SP levels are less repeatable toward the end of lactation. How has this been accounted for in the autumn bulk milk testing for *M. bovis*?

Screening bulk tank milk samples from April to June was intended to capture autumn herds as well as spring herds at the end of lactation. Physiological or epidemiological factors occurring at the end of lactation may improve our ability to detect *Mycoplasma bovis* antibodies in the sample, if present. All samples with an ELISA detect result from MilktestNZ were re-tested at MPI's Animal Health Laboratory, which gives us confidence in the repeatability and validity of the test result.

We are not certain of the effect of milk volume on the performance of the bulk tank milk ELISA. It could be argued that if truly infected animals are contributing to the vat, lower volumes would increase our ability to detect antibodies if present (i.e. a higher concentration of antibodies in the sample). We are working to collect extra data such as vat volume to analyse its influence on ELISA reactivity.

The concentration of fat in the sample may also affect the reactivity of the sample. MilktestNZ implemented an extra wash step as part of ELISA testing to remove more of the extra fat component observed for samples collected close to dry off. Once the true infection status of farms is determined through on-farm surveillance and testing, we can look at both if and how the performance of the ELISA changes through the lactation cycle and make adjustments if necessary.

**If we are to believe that the Autumn testing is as accurate as we are being told, why are there not more ELISA positive tests from the July sampling?**

Using the current bulk tank milk ELISA SP Ratio threshold, the number of detect results will be influenced by a number of factors including the number of tests performed. Through autumn we tested 34033 samples and for July we tested 1520 samples, partly explaining the difference in the number of ELISA detect results.

We have not before used the ELISA test to screen bulk tank milk samples over the April to June period. As such we didn’t have an expectation of the total number of ELISA detect results that would be reported. We imagine that there will be other factors influencing ELISA reactivity through the lactation cycle.

MPI’s Animal Health Laboratory performed re-testing of ELISA detect samples so that we could have confidence in the validity of the test results through the autumn period.

**MPI has predicted 8-12 new cases will be confirmed from bulk milk testing. How can this round of bulk milk testing produce approximately the same number of new cases even if there was ongoing spread or ‘background presence’ of *M. bovis*?**

This estimate was based on identifying factors that were associated with an increased ‘conversion-to-case’ rate for the Spring Surveillance Programme 2018. Here we observed that farms associated with more than one bulk tank milk ELISA detect result and/or farms
that were already known to the Programme due to animal movement risk events were more likely to be confirmed as infected properties. This estimate reflects the number of farms associated with either or both of these factors.

We will obviously only know the true number of confirmed cases once on-farm testing is completed. It is expected that the majority of confirmed cases will be dairies already undergoing Programme surveillance. Only a very small number of confirmed cases otherwise unknown to the Programme are expected.

**Given this is an active incursion Programme, why did MPI delay testing of bulk milk samples?**

At the conclusion of the Spring 2018 Bulk Tank Milk Surveillance Programme, a review of programme performance was essential to inform whether national bulk tank milk screening would continue and, if so, in what format. The *M. bovis* Programme consulted with our industry partners and DCANZ on the plan and worked together to implement the changes. While this process took place, samples were stored for later testing. The alternatives would have been to start testing without robust processes in place or not to have tested samples collected during the April to June 2019 period, potentially missing the detection of infected herds. We believe the approach taken was the best of all options.

Bulk tank milk samples were collected fortnightly from 15 April 2019. Collection continued for six fortnights. Samples for the first four fortnights were collected and stored in the freezer. On the 10 June 2019 when testing began, priority was given to test the freshly collected samples from fortnights five and six. Testing of the frozen samples was incorporated along with the testing of the fresh samples, starting with fortnight one and moving forwards. This priority was given to screen milking herds as close as possible to real-time and to screen for any true infection for autumn herds in early lactation.

**Why has it taken months to contact some farmers with known trace animals on their property?**

The Programme usually contacts farmers about trace animals far quicker (within one month of them being identified). There was a backlog and delay identified in April, but this is obviously not the standard.

Farmers that have a lower risk exposure (i.e. there are no trace animals on their farm) may not be contacted for a few months, while higher risk properties are prioritised.

Testing of bulk tank milk samples collected and stored since 15 April 2019 did not commence until 10 June 2019. Testing commenced once changes to the programme were implemented.

As samples over the April to June period had not been screened with the ELISA test before, we implemented extra test validation procedures to give us confidence in the repeatability of test results and to limit the number of false positive ELISA test results. Once a final test result was reported, farmers were contacted with urgent priority. While it was not ideal to
have a gap between date of sample collection and farmer notification, it was decided that this was preferable to not testing these herds at all.

Ongoing bulk tank milk screening, including the storage and testing of autumn samples, was publicly announced by MPI and communicated via Dairy NZ. MPI also worked with dairy companies to provide industry with communication messages for their own purposes.

The majority of farms with a bulk tank milk ELISA detect result will test negative for *Mycoplasma bovis* through on-farm sampling and testing. Any confirmed infected farms will have animal movements traced.

While it does make sense to prioritise testing of samples for herds where there is a suspicion that the herd may be *M. bovis* positive, I would think it would also make sense to prioritise testing of samples in a roughly North-South distribution to help identify possible *M. bovis* positive herds as soon as possible, relative to their calving date. This would minimise the potential for calves from potentially infected farms to be sold through sales.

Yes, in terms of minimising the potential for forwards transmission, it would be reasonable to prioritise testing in a roughly North-South distribution. However, due to the processes in place, it would have been procedurally complex to identify and pick out bulk tank milk samples based on geographic distribution from the stored and fresh samples. The testing laboratory has a high throughput and were able to complete the testing of all 34,033 samples by the end of July.

**How does the Programme prioritise testing of back-trace, forward-trace, and active properties?**

Testing of cattle on properties that *received* cattle or milk from Confirmed Properties during the time they were infected is prioritised over testing of cattle on properties that *supplied* cattle or milk to a Confirmed Property during the time it was infected. However, at some point testing of back-trace properties must be done and therefore sampling on these properties is scheduled in around sampling on forward-trace properties.

**What is the process for the communications team contacting trace properties to ask if trace animals are present? Does the team explain what ‘in-contact’ means?**

When properties of interest are identified through the tracing of cattle and milk movements, the *M. bovis* Casing team will make contact with the farmers to verify these movements as well as on-farm practices and animal contact information. The advisors explain why they are calling and seek information around trace animals and any animals that have had contact with the traces. ‘In-contact’ is explained so that the farmer understands.

**How does MPI tell a farmer they may have *M. bovis*, and ensure rational responses to questions about whether their farm needs to undergo testing?**
The Casing team is extremely sensitive to the information that is relayed to farmers and the impact this may have on the farmer during the initial casing call. We know that it is a stressful moment, and it is normal to have an emotional response. During this call we do reassure farmers that this does not mean their animals are infected but that we would like to do some on-farm blood sampling and testing to determine the infection status. The Casing team is trained in appropriate ways to carry out this conversation with farmers. In most cases, the information is gathered during the first call, but if there is the need for a further phone call the farmer is contacted at a more suitable time. If the Casing team member feels the farmer requires further support, contact information for the Rural Support Trust is given to the farmer and the farmer’s information is given to our Welfare team.

**Is MPI concerned that properties with high PCR positive groups have tested negative by their criteria on blood ELISA?**

The majority of groups of cattle that contained PCR positive animals were also herd-level ELISA (i.e. serology) positive. In some instances, trace-forward animals were PCR positive at slaughter, but the overall group was herd-level serology negative. In these instances, these groups are deemed to be infected and the property that they were on becomes a Confirmed Property. Groups that contained the PCR positive animal(s) are depopulated to remove the *M. bovis* infection risk all the cattle in the group present to the New Zealand cattle population.

**Is the same ELISA test being used for the bulk milk testing and the individual animal serum test? Is this ELISA used commercially in other countries? What company is this test produced by?**

The ELISA currently used in the *Mycoplasma bovis* Programme is a commercially available kit used in many other countries and is validated for the detection of antibodies against *M. bovis* in bovine serum, plasma or milk. It is produced by IDVet.

We use the same ELISA test for bulk tank milk testing and the animal serum test. It is produced by IDVet.

The Programme recently implemented changes to individual animal serum ELISA test thresholds and herd-level cut-offs. These changes were based on an analysis of a large number of IDVet ELISA test data accumulated through the Programme. These changes optimise the sensitivity and specificity of herd-level detection of *M. bovis* infection for the current situation in New Zealand.

**How well does the ELISA test perform?**

The IDVet ELISA test has been developed for the purposes of detecting antibody to *M. bovis*. Further, analysis of Programme data has shown that it is highly specific – 98% of individual serum samples from truly uninfected cattle test negative on IDVet ELISA. Further,
the IDVet ELISA test is quite sensitive at the individual animal level – 79% of individual serum samples from truly infected cattle ‘react’ on IDVet ELISA.

These findings show the IDVet ELISA is extremely accurate when it is used to test serum samples from a group of cattle, and the results are used to determine the *M. bovis* infection status of the whole group. There is no evidence of cross reactivity on the IDVet ELISA test.

**The MPI website states “there can be antibody cross-reactions with other bacteria normally found in New Zealand.” Please clarify what these bacteria are as this is a common question asked by farmers.**

The ELISA test that we use has been designed to be highly targeted towards *Mycoplasma bovis* antibodies. We do see a very small rate of false ‘detect’ or ‘react’ ELISA results. This is expected of any screening test. There is no evidence that antibodies to other bacteria found in New Zealand cross react on the ELISA test. It is highly unlikely that antibodies to *Leptospira spp.* or *Mycobacterium spp.* cross react on the *M. bovis* ELISA we use. This statement has been removed from the MPI website.

**Please outline the procedure for a bulk milk tank positive farm.**

The following protocol relates to farmers/farms associated with a bulk tank milk ELISA detect result in the absence of other risk events:

The farmer will be served a Notice of Direction (NOD) for the property where the milking herd, cattle deemed ‘in contact’ with them and calves are located that restricts cattle movements off the property.

A census will be conducted to allow us to identify any cattle of interest to the Programme.

**Sampling:**

Herd-level serological testing with the ELISA will be used to determine the infection status. Individual milk samples and nasal swabs for PCR testing will not be routinely collected.

Eligible animals for sampling are any milking animals currently contributing to the milk supply. If a split calving operation, and these herds are managed separately, each herd will be treated as a separate management group.

For management group(s) of ≤300 milking cows, a blood sample from a random sample of up to 220 cattle will be collected. For management group(s) of >300 milking cows, a blood sample from a random sample of 250 cattle will be collected.

In addition, a blood sample will be collected from all sick milking cows in the sick mob.

If the target sample sizes are achieved and the first round of on-farm serological testing is negative at a herd level, the movement control NOD may be lifted. Once
the census is complete and reviewed, no further surveillance for the bulk tank milk ELISA detect risk event in question will be required.

If the first round of on-farm serology testing is positive at the herd level, further rounds of on-farm sampling will be required. Subsequent sampling will not be targeted at previous reactors.

For a bulk tank milk ELISA detect risk event, cattle are only sent to slaughter if infection is confirmed at a herd level (i.e. two rounds of herd-level positive serology resulting in a Confirmed Property). If sent to slaughter, tonsillar swabs are collected for PCR testing.

**What is planned for ongoing surveillance of beef herds?**

Surveillance of commercial beef aggregating and finishing operations is ongoing. Routine monitoring of incoming feedlot cattle is underway, in addition to identification and evaluation of suspicious clinical cases by private veterinarians and incursion investigators. The expected prevalence of *M. bovis* outside the known network of infected properties is extremely low.

In the near future, background surveillance of the beef population may include the routine collection of abattoir samples from commercial beef herds, serum ELISA on properties identified as beef breeding operations and targeted syndromic surveillance.

The *M. bovis* Programme is working with its industry partners to identify population groups for sampling in a statistically meaningful manner, and to generate sampling plans that are minimally disruptive for beef producers.

**On average, how long does it take to clear false positive farms?**

The Programme has recently implemented substantial changes to how on-farm sampling and serological testing is conducted and interpreted. These changes mean that truly uninfected farms will move through the Programme a lot quicker.

The following answer applies to farms associated with a bulk tank milk ELISA detect result and no other risk events (i.e. trace animal movements). For truly uninfected farms, provided the required animals and the required number of animals are sampled, only a single round of sampling will be required. This requirement means that as soon as the Programme can conduct the sampling, perform the testing and interpret the test results, truly uninfected farms will have their status set to negative.

One requirement for farms associated with bulk tank milk ELISA detect results is to have a census undertaken so that we can identify any cattle of interest to the Programme. The requirement for a census is not tied to the lifting of any movement restrictions but must be conducted and reviewed before the farm can have their status set to negative. If the census and sampling are completed in a timely manner, truly uninfected farms may have their status set to negative within a month of movement restrictions being applied.
We have had a client where some of the animals which were serum ELISA positive (at initial testing) were ELISA negative when repeat tested at slaughter. Do you have any possible explanations?

Generally speaking, an individual animal’s ELISA test result does not change much across multiple sampling points. In instances where it does change there are many possible explanations, including the sensitivity of the IDVet ELISA and changes to *M. bovis* antibody levels in individuals over time.

On Confirmed Properties, individual animals that previously reacted to the IDVet ELISA test are targeted for slaughter sampling because analysis has shown that on Confirmed Properties it is these cattle that are most likely to produce tonsillar swabs that test PCR positive.

Some false positive results are expected with any sort of test. If monthly testing of bulk milk is ongoing, do you have any estimate of the number of farms which may be affected by unnecessary movement controls in the next few years?

Using the current ELISA SP Ratio thresholds, bulk tank milk screening is very specific, producing less than 0.5% false positive test results. For July 2019 we screened 1520 suppliers, two of which were reported with an ELISA detect result. For August 2019 we screened 8625 suppliers, 40 of which were reported with an ELISA detect result. It is difficult to provide an estimate as the number will largely depend on the number of tests conducted. Based on the number of screening tests conducted in 2018, it is expected that the number of tests will peak in September and October then substantially decline from November onwards. The number of ELISA detect test results are expected to follow a similar pattern.

The positive predictive value (i.e. the proportion of ELISA detect results associated with truly infected farms) of the bulk tank milk ELISA will depend on the threshold we use and the prevalence of infection in the national herd. As we identify and control infection as part of the Programme, the national prevalence will decrease with time, resulting in a larger proportion of false positive ELISA detect results. We will need to revise ELISA thresholds to minimise the number of false positive test results as we continue towards eradication.

How many infected properties are not presently connected to the *M. bovis* ‘network’?

At an operational level, this number is constantly changing. When any farm is first identified, the most likely infection pathway for that farm must be thoroughly investigated and all possible routes and linkages investigated. When a farm is identified as a ‘detect’ on bulk tank milk ELISA, the first step is to follow up the result of the screening test by doing on-farm testing. Should this on-farm testing return positive herd-level serology results and the farm progress to become a Confirmed Property, the farm is fully investigated. This investigation includes a census of animals present and reconciliation with NAIT records, in-depth interviews with the farmer, and culture of the *Mycoplasma bovis* present on the property so that the isolate can be whole-genome sequenced and fit into the genetic analysis and tree model.
The majority of our infected farms are found by following tracing links, based on information we have from NAIT, farmer interviews, NAIT tag censuses on other farms, and/or additional information we have collected in the course of investigation.

Gathering the totality of this information takes time and some farms are able to be resolved more simply than others. The linkages for some farms can be confirmed using only NAIT records but others can only be confirmed once whole-genome sequencing and molecular modelling have been confirmed.

At the Programme level, when all information we currently have is taken into account, evidence supports that all case farms are linked to the current network of infected farms. The evidence base includes tracing information from various sources, and genomic information gathered from *Mycoplasma bovis* isolates from the infected farms.

**When did cattle live imports to New Zealand cease?**

Live cattle imports from Australia ceased in 2013. Live cattle imports from other countries ceased in the late 1990s.

**One possible route of introduction of *M. bovis* into NZ is a live animal import. What investigative work is being done in relation to the movement of cattle from herds that have had imported cattle arrive into them in the past?**

MPI investigated all possible avenues, including back tracing of herds from cattle that arrived in New Zealand prior to the imposition of restrictions on the importation of live cattle.

The pathways by which *M. bovis* could have entered NZ are discussed in detail in the pathways report, and have been the subject of discussion by the Technical Advisory Group (TAG).

**What does MPI do about testing and decontaminating properties that have had known PCR positive animals on them?**

The Programme undertakes on-farm sampling and testing of cattle on properties where there are cattle that have been in contact with PCR-positive animals if those PCR-positive animals are known or suspected to have been infected at the time they were on those properties. These properties are collectively referred to as ‘back-trace properties’.

All Confirmed Properties are subject to the Programme’s cleaning and disinfection requirements.

**Why has MPI not used clinical veterinarians to speed the process?**

The *M. bovis* Programme has utilised clinical veterinarians since the beginning of the *M. bovis* response. We welcome and value the expertise clinical vets bring to our teams in Wellington and in the regions. At present, there are opportunities for vets to apply for new and existing roles in several locations. A key priority for our expanded Senior Regional
Veterinarian cohort will be to re-engage with private veterinary surgeons, the Veterinary Council of New Zealand and the New Zealand Veterinary Association.