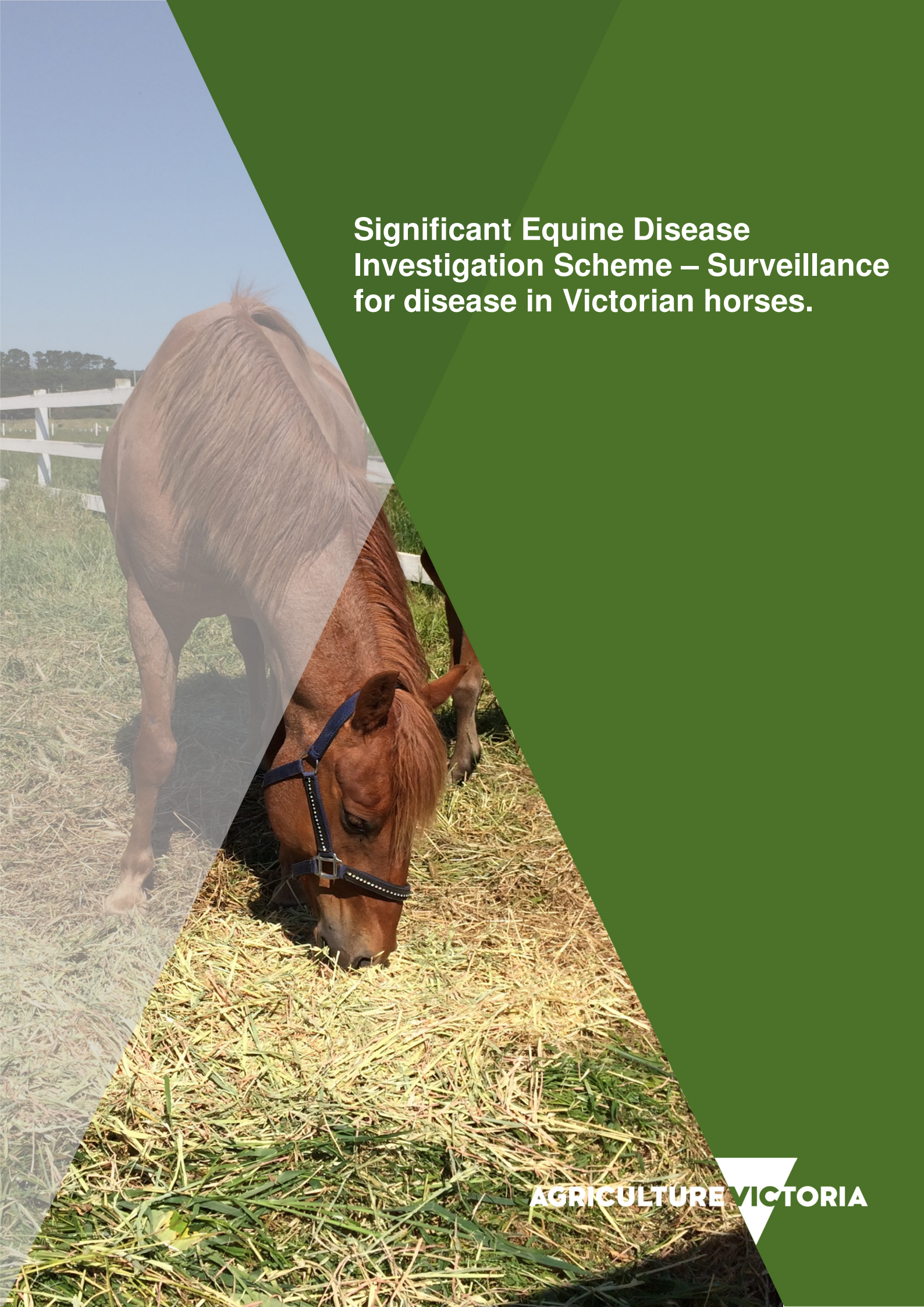




Significant Equine Disease Investigation Scheme – Surveillance for disease in Victorian horses.

AGRICULTURE VICTORIA

INTRODUCTION

Victoria is fortunate to be free from a range of important diseases of livestock that if present could have a detrimental impact on trade or human health. Early detection of emerging or exotic diseases is the key to their effective management. General surveillance is one component of Victoria's biosecurity effort. Private veterinarians play a key role in investigating and reporting outbreaks of significant disease in animals. Unfortunately, economic considerations may limit comprehensive investigations being undertaken. In order to reduce these barriers, the Victorian government introduced the Significant Disease Investigation (SDI) program which subsidised investigations into significant disease events.

In Victoria, however, investigations of disease events in horses have been under-represented in the SDI program in comparison to other livestock species.

The overall objective of the SEDIS project was to increase general surveillance capability and capacity in the equine sector by:

1. increasing the number of equine investigations undertaken and reported to the program by private veterinary practitioners, thus enhancing our ability to detect emerging and emergency diseases of horses.
2. improving the quality of equine disease investigations by ensuring that appropriate samples are collected to fully investigate the cause of disease in horses.
3. promoting effective collaboration between private veterinary practitioners, University of Melbourne veterinary school clinicians and government.

The project focused on three important disease syndromes in horses in Victoria; respiratory disease, abortion and neurological disease.

Respiratory disease is the second most common cause of lost training days in racehorses, abortion is a significant economic burden to the horse breeding industry and neurological disease is often associated with diseases of public health concern.

One of the outcomes of the work was to determine the infectious causes of these syndromes, and by exclusion, the non-infectious causes. In addition to the endemic infectious agents, exotic and emergency animal disease agents, also known to cause these syndromes, were included in the testing protocol.

The project was a collaboration between The University of Melbourne, Agriculture Victoria (Biosecurity and Agriculture Services) and Agriculture Victoria Research (AgriBio).

Methods

Private veterinarians were encouraged to participate in the project through direct contact with University of Melbourne clinicians and/or Agriculture Victoria veterinarians. They were supported to select appropriate cases and provide sufficient high-quality samples for analysis.

Horses that met the case definition for respiratory disease, abortion or neurological disease were investigated with no cost for laboratory testing as detailed in the project information sheet (Appendix 1). Testing was undertaken at the Centre for Equine Infectious Disease at the University of Melbourne, the state government laboratory (AgriBio) and the Australian Centre for Disease Preparedness (formerly AAHL) in Geelong. Both conventional and molecular microbiological techniques were used.

Respiratory disease

The testing regime for samples collected from animals displaying signs of respiratory disease included equine herpesviruses 1,2,4,5, equine picornaviruses (ERAV and ERBV), *Streptococcus equi* and equine viral arteritis. Nationally notifiable diseases such as equine influenza and EHV-1 were included in the testing panel performed on each sample. Hendra virus exclusion testing was also routinely conducted¹.

¹ Although a low probability, a differential diagnosis of Hendra should always be considered when examining horses with respiratory or neurological signs. Information for the safe management of suspected Hendra cases can be found at <https://www.business.qld.gov.au/industry/service-industries/veterinary-surgeons/guidelines-hendra/resources>

Abortion

The testing regime for samples collected from animals displaying signs of abortion included equine herpesvirus 1 and 4, as well as equine viral arteritis virus and *Chlamydia psittaci*. Histopathological examination was available where appropriate.

Neurological disease

The testing regime for samples collected from animals displaying signs of neurological disease included Hendra, EHV 1, Ross River virus (RRV), Murray Valley Encephalitis virus (MVEV) and West Nile -Kunjin virus (KUNV).

Stakeholder communications

To support the uptake of the project, information sheets and other communication materials were prepared. Project investigators presented information sessions to Agriculture Victoria animal health staff and the Horse Health Committee. Industry specific information sheets for private veterinarians and the Harness Racing Industry were distributed. The program was also flagged in the Australian Equine Journal and articles were submitted for inclusion in the Equine Veterinary Association and VetWatch newsletters.

The project began on 1 July 2018 and was expected to continue until 30 June 2020. Due to greater than expected participation the funds allocated to the project were completely expended with the last horses recruited in late October 2019.

Results

Sixty-eight investigations, involving 98 horses, were undertaken as part of the project. Fifteen horses were known to have died or been euthanised. The breakdown of investigations by syndrome was

- Neurological signs; 44 investigations involving 67 horses
- Abortion/stillbirth; 19 investigations
- Respiratory; two investigations involving 10 horses
- Mixed neurological/respiratory; two investigations involving two horses.

Figure 1 below shows the number of submissions received by month.

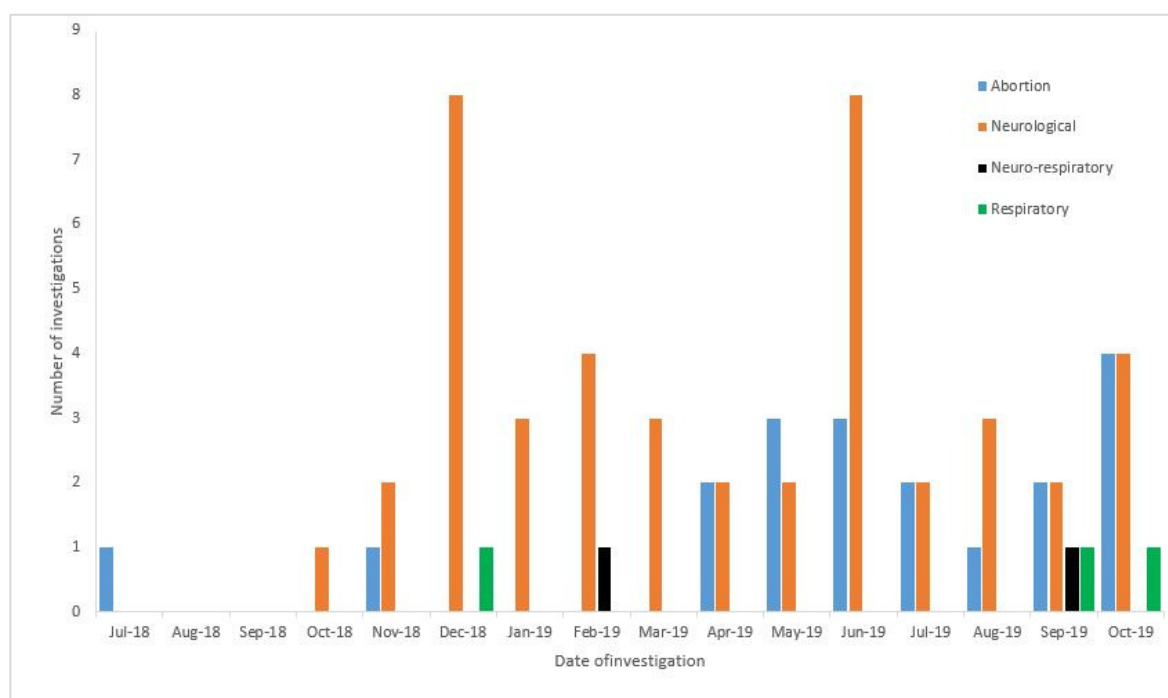


Figure 2 SEDIS submissions between 1 July 2018 and 31 October 2019 with colour indicating the main syndrome investigated

The location of investigations was reflective of the horse density across the state, with fewer horses, and therefore investigations, in the north west and alpine areas (Figure 2).

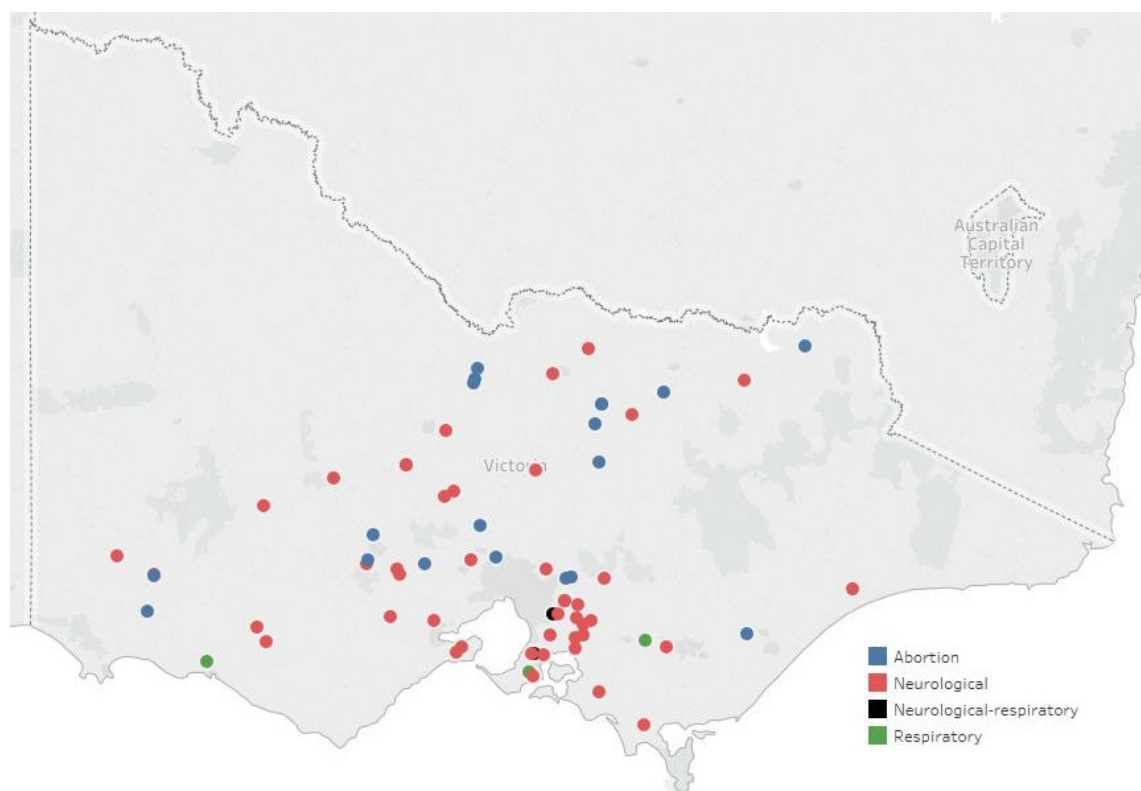


Figure 2 Geographic location of SEDIS investigations and syndrome investigated

Table 1 Summary of case results by syndrome

Syndrome	Result	Number of investigations
Abortion	<i>C. psittaci</i>	3
	EHV1	1
	Placentitis (agent undetermined)	4
	Undetermined	11
Neurological	Cryptococcus sp	1
	EHV1	2
	Undetermined	41
Respiratory	EHV5	1
	EHV5, EHV4, ERB	1
	Undetermined	1
Neuro-respiratory	EHV5 & EHV2	1
	ERB	1
Total number of cases		68

EHV: Equine Herpes Virus

ERB: Equine rhinitis B virus

Neurological Investigations (Table 1)

Of the 67 horses tested

- No Hendra was detected.
- Two horses were diagnosed with EHV1, one of which died.
- *Cryptococcus* sp was detected in the CSF of a third deceased horse (the only case to submit CSF).
- There was no evidence of recent RRV or flavivirus infection in any of the horses (where tested).
- The cause of the clinical signs in 41 (64 horses) investigations could not be determined.

In addition to the routine testing (that had returned negative results), New Generation Sequencing (NGS) was performed on samples from two investigations.

- ❖ Blood samples from six horses showing signs of acute disease from an outbreak on the Bellarine Peninsula were tested with no agent detected. The horses had displayed lethargy, inappetence and ventral oedema.
- ❖ Next Generation Sequencing (NGS) detected *Cryptococcus* sp in the CSF of a horse from south Gippsland

Abortion Investigations (Table 1)

Of the nineteen abortion investigations undertaken

- *Chlamydia psittaci* was identified as the cause of abortion in three horses. Two of the cases were paddock mates, located in the north east. They aborted less than three weeks apart. The third case occurred on a separate property also located in the north east.
- EHV1 was identified as the cause of abortion in another case.
- Placentitis of unknown aetiology was found in four other cases.
- The cause of the abortion in eleven cases could not be determined.

Respiratory and neuro-respiratory investigations (See Table 1)

- Of the three respiratory and two neuro-respiratory respiratory investigations
 - No equine influenza was detected
 - EHV 5 was detected in all five horses from a single outbreak on one property.
 - Equine rhinitis virus B was the only agent detected in a horse that was subsequently euthanised.
 - A range of other equine viruses, (Equine rhinitis virus B, EHV 4, EHV 5 and EHV 2) were detected in combination making their significance difficult to determine.

Conclusion

The project reached a large number of equine veterinarians who showed keen interest. Our aim will be to maintain the links with these veterinarians and encourage submission of investigations through the general SDI program. Engaging the larger industry players in the program proved difficult.

The number of submissions was higher than previous years (excluding the years of disease outbreaks associated with strangles and arboviruses).

For the first time in Victoria *C.psittaci* was identified as a cause of abortion. (The case has been written up in the Australian Veterinary Journal).

Of interest was the use of NGS technology; testing that would normally not be undertaken. This testing identified *Cryptococcus* sp as the cause of neurological disease and identified the value of collecting CSF (where possible) as an aid in diagnosis.



Significant Equine Disease Investigation Scheme (SEDIS)



The University of Melbourne and Agriculture Victoria are investigating outbreaks of respiratory disease (including strangles), abortion and neurological disease in Victorian horses.

Background:

In Victoria, investigations of disease events in horses are under-represented in the Significant Disease Investigation (SDI) program in comparison to other livestock species. This project will facilitate the investigation of outbreaks of three important disease syndromes in horses in Victoria using conventional and molecular microbiological techniques in order to determine the infectious, or other, cause(s).

Is this horse eligible?

A horse will be eligible for inclusion in the study if it resides in Victoria at the time of illness and meets one of the following case definitions:

Outbreak of respiratory disease in racing horses:

- Two or more horses are concurrently affected; **AND**
- There is evidence that the disease is spreading within the stable; **AND**
- Affected horses have a combination of more than one of the following clinical signs:

o Fever (Temperature $>38.5^{\circ}\text{C}$)	o Coughing
o Nasal discharge (copious serous-mucopurulent)	o Inappetance
o Rales, or other abnormal respiratory sounds on auscultation	

Samples will be tested for the presence of equine influenza, equine herpesviruses 1, 2, 4 and 5, equine picornaviruses (ERAV and ERBV), *Streptococcus equi* and equine viral arteritis.

Any case of abortion:

All equine abortions are eligible, with an abortion defined as:

- any mare with a previously confirmed pregnancy diagnosis that loses the pregnancy; **AND**
- conceptus (foetus and placenta) is recovered and samples collected for laboratory testing.

Samples will be tested for equine herpesvirus 1 and 4, equine viral arteritis virus and *Chlamydia psittaci*, as well as EAFL or other placental pathologies.

Any case of neurological disease:

A horse is eligible if it is observed with one or more of the following clinical signs:

o Ataxia	o Depression
o Altered mental state	o Pyrexia
o Muscle fasciculations	o Facial paralysis
o Hypermetria	o Blindness
o Hyperaesthesia	o Lameness
o Circling	o Oedema
o Recumbency	o Colic
o Weight loss	o Seizures

Samples will be tested for EHV 1, Hendra¹, as well as exposure to Ross River virus, Murray Valley Encephalitis virus and West Nile virus (Kunjin strain).

¹ Although a low probability, a differential diagnosis of Hendra should always be considered when examining horses with respiratory or neurological signs. Information for the safe management of suspected Hendra cases can be found at <https://www.business.qld.gov.au/industry/service-industries/veterinary-surgeons/guidelines-hendra/resources>



Significant Equine Disease Investigation Scheme (SEDIS)



How you can participate?

Before any samples are taken, please contact your local AgVic district veterinarian to discuss inclusion of the horse(s) in this research study. This project will continue to accept samples until **30 June 2020**. SDI subsidies are available where the necessary conditions are met (<http://agriculture.vic.gov.au/agriculture/pests-diseases-and-weeds/animal-diseases/disease-surveillance-programs/significant-disease-investigation-sdi-program>).

Miscellaneous investigations:

Laboratory costs associated with other (miscellaneous) investigations, where the above case definitions do not apply, may be funded by Agriculture Victoria if the investigation meets the requirements of the general SDI scheme. Please contact your local AgVic veterinarian to determine whether your case may be eligible.

Samples to be collected:

Outbreak of respiratory disease:

- Clotted blood (serum) for acute and convalescent serology²; **AND**
- EDTA blood for pathogen testing; **AND**
- Nasopharyngeal swab samples (as required).

[Broncho-alveolar lavage or trans-endoscopic tracheal lavage sample may be collected, but only after consultation with The Centre for Equine Infectious Disease (The University of Melbourne; contact details below)]

Any case of abortion:

Please **DO NOT** send the complete foetus and membranes.

- Clotted blood from the mare for acute and convalescent serology²; **AND**
- Fresh and fixed samples from the placenta (2 x 2cm); **AND**
- Fresh and fixed foetal tissue samples: liver, lung, spleen and thymus.

NOTE: Sterile sample pots must be used for fresh tissue. It is recommended that samples from the aborted conceptus be collected using appropriate personal protective equipment as several equine abortigenic pathogens are zoonotic.

Any case of neurological disease:

- Clotted blood for acute and convalescent serology²
- 2 x EDTA blood

Samples **must** be submitted using the project-specific lab submission form (accessible on The Agriculture Victoria VetSource webpages(www.vic.gov.au/vet-source)).

Diagnostic test results:

The submitting veterinarian will be provided with a comprehensive set of results at no cost. A pre-defined list of syndrome-specific diagnostic tests will be undertaken; the completion of any additional tests is at the discretion of The Centre for Equine Infectious Disease.

If you have any questions, please contact your local AgVic veterinary officer or:

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² An individual horse will need to be re-sampled 2 weeks (+/- 2 days) after the initial visit (i.e. acute and convalescent samples).

Appendix 2

Table 2 Summarised test results for horses that have aborted or displayed neurological or respiratory signs.

	Neurological		Abortion		Respiratory		Neuro-respiratory	
	Number tested	Number positive	Number tested	Number positive	Number tested	Number positive	Number tested	Number positive
Hendra	65*	0	0	0	1	0	2	0
EHV 1	65*	2	19	1	6	0	2	0
RRV	40	0	0	0	0	0	1	0
Flavivirus	43	0	0	0	0	0	1	0
Cryptococcus	1	1						
<i>Chlamydia psittaci</i>	0	0	18	3	0	0	0	0
Equine rhinitis B virus	0	0	0	0	5	1	2	1
<i>S equi equi</i>	0	0	0	0	10	1**	2	0
Equine influenza	0	0	0	0	10	0	2	0
EHV 4	0	0	19	0	10	1	0	0
EHV 5	1	0	0	0	10	7	2	1
Equine viral arteritis	13	0	8	0	10	0	2	0

*2 samples not tested, ** Evidence of past infection

