Final Report

Summary

Diagnostic investigations into Chlamydia psittaci pathology in equine abortion
Objectives

Characterise at the microscopic level the effects of infection with Chlamydia psittaci on equine foetuses/foals and the placenta:

- Compare microscopic methods and DNA-based methods to identify which organs are targeted by Chlamydia psittaci to guide sampling protocols
- Develop rapid and accurate point-of-care DNA-based tests for the detection of two major infectious agents causing reproductive loss in horses - Chlamydia psittaci and equine herpesvirus-1.

Background and Project Importance

Bacterial and viral diseases are major causes of reproductive loss in horses, either through abortion events or the death of newborn foals. Chlamydia psittaci is an emerging bacterial cause of reproductive loss in horses only recently described in Australian equines, and yet, during a recent outbreak over 20% of lost foals were attributed to this organism. Equine herpesvirus-1 (EHV-1) causes respiratory disease in adult horses, is highly contagious and is the major viral cause of equine abortion in Australia.

Together EHV-1 and C.psittaci have a significant economic impact on Australian Thoroughbred breeders. C.psittaci is also a risk to veterinarians and stud workers coming into contact with infected abortion products and can cause life-threatening pneumonia. This project aims to address these issues by providing clear diagnostic criteria for C.psittaci and rapid diagnostic tests for use at the point-of-care.

Results and Outcomes to date

In this project, 55 cases of foetal/foal loss were examined using laboratory DNA testing for C. psittaci and microscopy to determine how C. psittaci causes disease. DNA testing on the different organ tissues from aborted foetuses revealed that the placental tissues and the foetal lung (Figure 1) contained the highest number of infectious particles.

These findings were confirmed microscopically, with placental tissues being those most obviously affected by the infection (Figure 2). Inflammation of the placental tissues was the main microscopic finding associated with C. psittaci infection and could be distinguished from the type of inflammatory response seen as a result of other infectious causes of abortion. C. psittaci in the lung appeared to arise from inhalation by foetuses of infected amniotic fluids (Figure 3). On the basis of these findings we established diagnostic criteria for C. psittaci abortion:

- examination of foetus and placenta
- a positive result from a DNA-based test
- lymphohistiocytic placentitis with a special stain used to confirm C. psittaci
- exclusion of other causes of equine abortion, particularly EAFL, EHV and leptospirosis.

Figure 1 Graph showing the number of C. psittaci particles detected in pooled foetal compared to pooled placental tissues. Pooled foetal tissues consisted of lung, liver, thymus and spleen samples. Placental tissues had a significantly higher number of chlamydial particles compared to foetal tissues but foal lung samples also contained very high particle numbers.

Figure 2 Microscopic examination of an amnion from an abortion case with a special stain for C. psittaci. The cells are stained blue and the lining of the amnion is stained red indicating the presence of C. psittaci.

Figure 3 Microscopic examination of a foal lung with a special stain for C. psittaci. Lung cells are blue and red staining indicates the presence of C. psittaci in the air spaces of the lung.
The project successfully developed two rapid DNA-based tests for equine herpesvirus-1 (EHV-1) and Chlamydia psittaci which require only rudimentary equipment and can be used at the point-of-care. Both tests were highly specific for their target disease agent and the results compared well to laboratory-based tests in terms of their detection sensitivity. The results of these DNA-based tests can be interpreted with the naked eye using a simple colour change reaction (see Figure 4) and the tests have been adapted to include a rapid sample processing step, reducing total testing time to approximately 45 minutes.

**Implications and recommendations**

The diagnostic criteria and microscopic characteristics of C. psittaci abortion have been defined, allowing veterinary pathologists to diagnose the disease with more confidence, thus benefiting veterinarians and affected breeders (scientific paper in preparation - final report to be published).

The rapid diagnostic tests developed in this study will benefit veterinarians by providing timely (same day) diagnosis and allowing them to give timely advice to affected breeders. This will ensure more rapid treatment of affected horses and earlier interventions to protect unaffected horses on the same farm.

We recommend that these point-of-care tests are implemented where possible with follow up laboratory confirmation. Placental and lung tissues are the preferred tissue types to maximise the sensitivity of DNA-based tests.

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