



Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Akter, R;Stent, AW;Sansom, FM;Gilkerson, JR;Burden, C;Devlin, JM;Legione, AR;El-Hage, CM

Title:

Chlamydia psittaci: a suspected cause of reproductive loss in three Victorian horses

Date:

2020-08-23

Citation:

Akter, R., Stent, A. W., Sansom, F. M., Gilkerson, J. R., Burden, C., Devlin, J. M., Legione, A. R. & El-Hage, C. M. (2020). Chlamydia psittaci: a suspected cause of reproductive loss in three Victorian horses. AUSTRALIAN VETERINARY JOURNAL, 98 (11), pp.570-573. <https://doi.org/10.1111/avj.13010>.

Persistent Link:

<https://hdl.handle.net/11343/276180>

Title: *Chlamydia psittaci*: a suspected cause of reproductive loss in three Victorian horses

Running title: *C. psittaci* and equine abortion in Victoria

Rumana Akter^{a,b}, Andrew W. Stent^c, Fiona M. Sansom^a, James R. Gilkerson^a, Chelsie Burden^d, Joanne M. Devlin^{a,*^}, Alistair R. Legione^{a^} and Charles M. El-Hage^{a^}

^a Asia Pacific Centre for Animal Health, The Melbourne Veterinary School, The University of Melbourne, Parkville, Victoria, 3010 Australia

^b Department of Medicine (RMH), The University of Melbourne, Parkville, Victoria, 3010 Australia

^c The Melbourne Veterinary School, The University of Melbourne, Werribee, Victoria, 3030 Australia

^d Goulburn Valley Equine Hospital, 905 Goulburn Valley Hwy, Congupna, Victoria, 3633 Australia

[^]These authors contributed equally

*Corresponding author: devlinj@unimelb.edu.au

Acknowledgements

We are grateful to Kim Jeffers (The University of Melbourne) for extracting DNA and Agriculture Victoria for support and valued assistance.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1111/avj.13010](https://doi.org/10.1111/avj.13010)

Abstract

Chlamydia psittaci was detected by PCR in the lung and equine foetal membranes of two aborted equine foetuses and one weak foal from two different studs in Victoria, Australia. The abortions occurred in September 2019 in two mares sharing a paddock north east of Melbourne. The weak foal was born in October 2019 in a similar geographical region and died soon after birth despite receiving veterinary care. The detection of *C. psittaci* DNA in the lung and equine foetal membranes of the aborted or weak foals, and the absence of any other factors that are commonly associated with abortion or neonatal death, suggest that this pathogen may be the cause of the reproductive loss. The detection of *C. psittaci* in these cases is consistent with the recent detection of *C. psittaci* in association with equine abortion in New South Wales. These cases in Victoria show that *C. psittaci*, and the zoonotic risk it poses, should be considered in association with equine reproductive loss in other areas of Australia.

Keywords: *Chlamydia psittaci*, equine, abortion, Victoria

Case report introduction

Chlamydia psittaci causes a zoonotic disease known as psittacosis ¹. The disease in humans is characterized by fever, malaise, myalgia and atypical pneumonia ². Birds are commonly infected and a major feature of *C. psittaci* infection in birds is its ready transmission to mammalian species, including humans ^{3,4}. Human psittacosis is frequently reported worldwide and mainly occurs as a result of direct or indirect interactions with infected birds or bird excreta ^{2,5,6}.

In addition to humans, *C. psittaci* has been detected in other mammals including ruminants and horses ⁷⁻¹⁰. In animals, respiratory, intestinal, arthritic and reproductive diseases can occur due to *C. psittaci* infection ¹¹. Horses are considered to be occasional hosts of *C. psittaci* and infection can result in pneumonia, conjunctivitis, polyarthritis or abortion ^{9,11}. Until recently, *C. psittaci* infection has often been overlooked amongst the differential diagnoses of equine reproductive loss, with only limited epidemiological studies conducted to examine the associations between *C. psittaci* and equine abortion. Within this last decade however, several reports of *C. psittaci* as the aetiological agent of equine abortion have been published ¹¹⁻¹⁵. These reports have included the description of *C psittaci*

infection in cases of equine abortions and/or neonatal illness, with associated cases of human disease, in New South Wales, Australia.

In this case series, we report three cases of equine abortion or neonatal death associated with detection of *C. psittaci* in foetal and/or equine foetal membranes. To our knowledge these are the first reports of Australian equine abortions/reproductive loss in association with *C. psittaci* outside of New South Wales and highlights the importance of considering *C. psittaci*, and the zoonotic risk that it presents, in association with equine reproduction loss in other areas of Australia.

Clinical features

History and clinical findings

A 7-year-old Friesian mare aborted a 9.5 month old foetus (Foetus ID 3341) in September 2019. Two weeks later another mare (also Friesian) residing in the same paddock also aborted (Foetus ID 3345). The gestational age of mare and the age of foetus in the second abortion event were not recorded. The vaccination status of the mares was unknown. No predisposing factors for abortion were identified.

In October 2019, a thoroughbred mare delivered a weak foal (Foal ID 3350) which died 24 hours later despite resuscitative treatment for suspected neonatal isoerythrolysis. The one-day-old thoroughbred foal displayed clinical signs of disease consistent with neonatal isoerythrolysis, including weakness, bradycardia, bradypnoea, low temperature (35°C), congested mucous membranes and cold extremities. The two farms were located North East of Melbourne, 75 km apart from each other.

Diagnostic sample collection and DNA extraction

Foetal tissues (lung, liver, thymus, spleen and equine foetal membranes) were initially submitted to the AsiaPacific Centre for Animal Health (APCAH) in Melbourne, Australia for equine herpesvirus-1 (EHV-1) and 4 (EHV-4) testing using PCR. Following negative EHV-1 and EHV-4 results the samples were tested for the presence of *C. psittaci* DNA. For this, DNA from individual tissues was extracted by a Kingfisher robot with a MagMAX™ Core Nucleic Acid Purification Kit (Thermo Fisher Scientific)

according to the manufacturer instructions. Extracted DNA was then stored at -80°C before being used as template in a PCR to detect *C. psittaci*.

C. psittaci screening and genotyping

Individual foetal tissues were tested by targeting a 460 bp region of the 16S rRNA gene of *Chlamydiaceae* using quantitative PCR as described previously¹⁶. The PCR was performed in a total volume of 25 µL containing 1× Green GoTaq Flexi Buffer (Promega), 2 mM MgCl₂ (Promega), 0.2 mM of each deoxynucleotide triphosphate (Bioline), 2 µM of each primer (16SG-F TGATGAGGCATGCAAGTC, 16SG-R TTACCTGGTACGCTCAAAT), 1.5 U of GoTaq DNA polymerase (Promega), 10 µM of SYTO 9 fluorescent nucleic acid stain (Invitrogen) and 5 µL DNA template, with Milli-Q filtered water to reach final volume. Positive control reactions used DNA extracted from the target sequences of *C. psittaci* and *C. pecorum* cloned into pGEM-T (Promega). Milli-Q filtered water was used as template in negative control reactions. The limit of the assay was determined by testing ten-fold serial dilutions from 10⁸-10¹ copies of the targeted plasmid in triplicate. Thermal cycling conditions were the same as described previously¹⁶. The curves produced from control samples of *Chlamydia* DNA were used to compare the profile of the melting curve for each clinical sample. Samples were considered to be positive if the Ct value was < 35 and the profile of the melt curve was consistent with those generated from positive control samples. The genome copies of *Chlamydia* in the reactions were quantitated from the qPCR results.

Multi-locus sequence typing (MLST) was performed on *C. psittaci* positive samples according to the previously developed scheme by Pannekoek et al¹⁷. Fragments of seven housekeeping genes (*gatA*, *oppA*, *hflX*, *gidA*, *enoA*, *hemN* and *fumC*) of *Chlamydiales* were amplified and sequenced using the oligonucleotide primers previously published on the *Chlamydiales* MLST website (https://pubmlst.org/chlamydiales/info/MLST_primers_2012.pdf)¹⁸. The PCR of each targeted gene was performed as described previously¹⁹. All PCRs were performed in a T100 Bio-Rad thermal cycler. The amplified PCR products (381 – 635 bp in length) were visualised by UV transillumination after agarose gel electrophoresis.

PCR products were purified from the PCR reaction mixtures using the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's instruction and eluted in 30 µL Tris-EDTA buffer. The quantity of purified DNA was estimated using a spectrophotometer (NanoDrop Technologies) and sequenced using Big Dye Terminator (BDT) v3.1 (Life Technologies) according to manufacturer's instructions. The purified products were sent to Australian Genome Research Facility (AGRF) for capillary separation. Geneious bioinformatics software version 11.1.4 (Biomatters) was used to trim, manually curate and align all obtained sequences²⁰. Nucleotide sequences were compared with publicly available sequences in the Genbank database²¹ using the NCBI Nucleotide Basic Local Alignment Search Tool (BLASTN) online algorithm²². The online *Chlamydiales* MLST database (<https://pubmlst.org/chlamydiales>) was used to determine the allelic designation for each locus of the *C. psittaci* sequences, and the sequence types (STs) were identified using the subsequent allelic profile. The alignment of concatenated MLST fragments was used to generate a phylogenetic tree. A phylogenetic tree with 500 bootstraps and GTR+I model was generated with PhyML²³ using multiple sequence MAFFT²⁴ alignment of positive strains, as implemented in Geneious.

Results and Discussion

C. psittaci DNA was detected by qPCR in lung and equine foetal membrane samples from foetus ID 3341, in the equine foetal membranes from foetus ID 3345 and in the lung from foal ID 3350. Non-infectious causes of abortion such as twin pregnancy, foetal malformations, umbilical cord strangulations and uterine torsion were not observed. The presence of *C. psittaci*, the exclusion of other pathogens (EHV-1 and EHV-4 were not detected by PCR) and the absence of any apparent non-infectious causes of abortion in these three cases suggests that *C. psittaci* was the cause of the abortion or foal death and indicates that this pathogen is a threat to equine industries beyond NSW in Australia where it has been detected previously. The load of *C. psittaci* in positive samples (mean = 1.27×10^7 genome copies/µL of extract) was comparable to the highest levels detected in foetal tissue in an epizootic of *C. psittaci* equine reproductive loss in NSW¹⁴ further suggesting that *C. psittaci* was the causative agent, rather than an incidental detection, in these Victorian cases.

Previous studies have used histopathological examination to confirm *C. psittaci* infection in association with cases of equine abortion or reproductive loss¹⁴. Histopathological analysis was attempted in

these current cases, however the tissues had been stored at -70°C without any fixative and the cellular detail was insufficiently preserved for detection of lesions. Investigation of future cases of equine abortion events would be aided by histopathological examination of appropriately stored tissues, but it is important to note that in previous reports of *Chlamydia*-associated abortion in mares, 31% of positive cases did not display any histological lesions, while a further 20% only displayed mild lymphohistiocytic placentitis that could easily be masked by autolysis¹¹. These findings indicate that routine histological assessment is not a sensitive method for detection of *C. psittaci* infection, and other ancillary testing should be used to support the diagnosis.

When MLST analysis was applied to the *C. psittaci* positive samples they were identified as belonging to ST24. This same ST has previously been detected in humans, Australian parrots, including wild parrots in Victoria, and other *C. psittaci* equine abortion cases in Australia.²⁵⁻²⁸ (Figure 1). The findings from the molecular analyses conducted in this case report are consistent with Australian native parrots being the reservoir of equine *C. psittaci* infection in Australia^{14, 25, 26}. Information relating to direct or in-direct contact between Australia native parrots and the horses in this case report is not available. A greater number of Australian white ibis (*Threskiornis Molucca*) in the area had been noted during the spring of 2019 by one of the authors (CB). *C. psittaci* have been detected in species of ibis in Europe,²⁹ but to date the ST24/6BC genotype has not been specifically detected.

The highly virulent 6BC clade of *C. psittaci* detected in equine abortion samples in Australia is a concern for public health. Recently, human *C. psittaci* infections have been reported after contact with equine foetal membranes within an Australian veterinary school and hospital in NSW. The cases identified in this report were at least 200 km south of the southernmost NSW property, and the first reported in Victoria^{14, 30}. Measures such as the use of appropriate personal protective equipment and biosecurity procedures are recommended to decrease the risk of human infection. These actions may also help in preventing spread of infection among horse populations.

Conclusion

This report adds to the previous detections this decade of equine psittacosis in New South Wales and highlights the importance of including *C. psittaci* in routine diagnostic testing in cases of equine

abortion or reproductive loss across different regions of Australia. As *C. psittaci* is zoonotic this report also underscores the importance taking appropriate protective measures when dealing with cases of equine abortion or reproductive loss in order to avoid human infection and disease.

Conflict of interest

The authors declare that they have no competing interests.

References

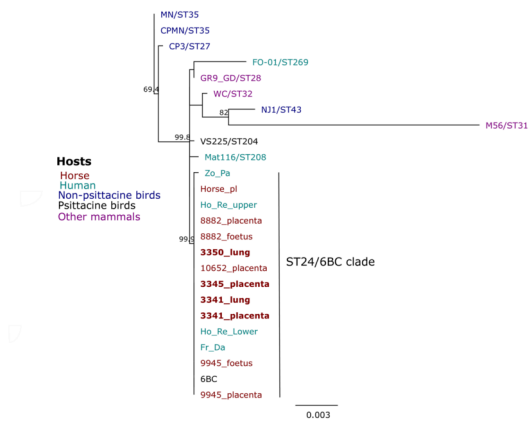
1. Kaleta EF, Taday EMA. Avian host range of *Chlamydophila* spp. based on isolation, antigen detection and serology. *Avian pathology* 2003;32:435-462.
2. McGuigan CC, McIntyre PG, Templeton K. Psittacosis outbreak in Tayside, Scotland, December 2011 to February 2012. *Eurosurveillance* 2012;17:20186.
3. Knittler MR, Sachse K. *Chlamydia psittaci*: update on an underestimated zoonotic agent. *Pathogen and disease* 2015;73:1-15.
4. Pal M. *Chlamydophila Psittaci* as an emerging zoonotic pathogen of global significance. *International Journal of Vaccines and vaccination* 2017;4:00080.
5. Gaede W, Reckling KF, Dresenkamp B et al. *Chlamydophila psittaci* infections in humans during an outbreak of psittacosis from poultry in Germany. *Zoonoses public health* 2008;55:184-188.
6. Laroucau K, De Barbeyrac B, Vorimore F et al. Chlamydial infections in duck farms associated with human cases of psittacosis in France. *Veterinary microbiology* 2009;135:82-89.
7. Lenzko H, Moog U, Henning K et al. High frequency of chlamydial co-infections in clinically healthy sheep flocks. *BMC veterinary research* 2011;7:29.
8. Longbottom D, Coulter LJ. Animal chlamydioses and zoonotic implications. *Journal of comparative pathology* 2003;128:217-244.
9. Theegarten D, Sachse K, Mentrup B et al. *Chlamydophila* spp. infection in horses with recurrent airway obstruction: similarities to human chronic obstructive disease. *Respiratory research* 2008;9:14.
10. Van Loo H, Pardon B, De Schutter P et al. Detection of *Chlamydia psittaci* in Belgian cattle with signs of respiratory disease and milk drop syndrome. *Veterinary record* 2014;175:562-562.
11. Szeredi L, Hotzel H, Sachse K. High prevalence of chlamydial (*Chlamydophila psittaci*) infection in fetal membranes of aborted equine fetuses. *Veterinary research communications* 2005;29:37-49.
12. Henning K, Sachse K, Sting R. Demonstration of *Chlamydia* from an equine abortion. *DTW Deutsche tierärztliche Wochenschrift* 2000;107:49-52.
13. Polkinghorne A, Greub G. A new equine and zoonotic threat emerges from an old avian pathogen, *Chlamydia psittaci*. *Clinical microbiology and infection* 2017;23:693-694.

14. Jenkins C, Jelocnik M, Micallef ML et al. An epizootic of *Chlamydia psittaci* equine reproductive loss associated with suspected spillover from native Australian parrots. *Emerging microbes and infections* 2018;7:88.
15. Taylor KA, Durrheim D, Heller J et al. Equine chlamydiosis—An emerging infectious disease requiring a one health surveillance approach. *Zoonoses and public health* 2018;65:218-221.
16. Robertson T, Bibby S, O'Rourke D et al. Characterization of *Chlamydiaceae* species using PCR and high resolution melt curve analysis of the 16S rRNA gene. *Journal of applied microbiology* 2009;107:2017-2028.
17. Pannekoek Y, Dickx V, Beeckman DSA et al. Multi locus sequence typing of *Chlamydia* reveals an association between *Chlamydia psittaci* genotypes and host species. *Plos one* 2010;5:e14179.
18. Pannekoek Y JKA, Bray J.E, Maiden M.C. *Chlamydiales* PubMLST Database. Wellcome Trust, University of Oxford, Oxford, UK. Retrieved 01/14/2020 from <https://pubmlst.org/chlamydiales/>. 2010.
19. Amery-Gale J, Legione AR, Marends MS et al. Surveillance for *Chlamydia* spp. with multilocus sequence typing analysis in wild and captive birds in Victoria, Australia. *Journal of wildlife diseases* 2020;56:16-26.
20. Kearse M, Moir R, Wilson A et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 2012;28:1647-1649.
21. Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. 2015;44:D67-D72.
22. Boratyn GM, Camacho C, Cooper PS et al. BLAST: a more efficient report with usability improvements. *Nucleic acids research* 2013;41:W29-W33.
23. Guindon S, Dufayard J-F, Lefort V et al. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic biology* 2010;59:307-321.
24. Katoh K, Misawa K, Kuma Ki, Miyata T. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic acids research* 2002;30:3059-3066.
25. Jelocnik M, Branley J, Heller J et al. Multilocus sequence typing identifies an avian-like *Chlamydia psittaci* strain involved in equine placentitis and associated with subsequent human psittacosis. *Emerging microbes & infections* 2017;6:e7.

26. Branley J, Bachmann NL, Jelocnik M, Myers GSA, Polkinghorne A. Australian human and parrot *Chlamydia psittaci* strains cluster within the highly virulent 6BC clade of this important zoonotic pathogen. *Scientific reports* 2016;6:30019.
27. Amery-Gale J, Legione AR, Marenda MS et al. Surveillance for *Chlamydia* spp. with multilocus sequence typing analysis in wild and captive birds In Victoria, Australia. *Journal of wildlife diseases* 2019.
28. Sutherland M, Sarker S, Vaz PK et al. Disease surveillance in wild Victorian cacatuids reveals co-infection with multiple agents and detection of novel avian viruses. *Veterinary microbiology* 2019;235:257-264.
29. Vorimore F, Hsia R-c, Huot-Creasy H et al. Isolation of a new *Chlamydia* species from the feral sacred ibis (*Threskiornis aethiopicus*): *Chlamydia ibidis*. *Plos one* 2013;8.
30. Chan J, Doyle B, Branley J et al. An outbreak of psittacosis at a veterinary school demonstrating a novel source of infection. *One health* 2017;3:29-33.

Figure Legends

Figure 1: PhyML phylogenetic tree generated with 500 bootstraps using the alignment of the concatenated MLST fragment sequences (3098 bp) of Australian and international strains of *C. psittaci*. *C. psittaci* sequences from this study are shown in bold. The host species is denoted by different colours. Bootstrap values greater than 60 are shown on the tree nodes. Previously published sequences were retrieved from the MLST database ¹⁸.



AVJ_13010_Figure 1 600dpi.tiff