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Original article

Challenges in using serological methods to explore historical transmission risk of *Chlamydia psittaci* in a workforce with high exposure to equine chlamydiosis

Belinda Jones, Kathryn Taylor, Robyn M Lucas, Tony Merritt, Catherine Chicken, Jane Heller, Joan Carrick, Rodney Givney and David N Durrheim

Abstract

This report describes the challenges encountered in using serological methods to study the historical transmission risk of *C. psittaci* from horses to humans.

Methods

In 2017, serology and risk factor questionnaire data from a group of individuals, whose occupations involved close contact with horses, were collected to assess the seroprevalence of antibodies to *C. psittaci* and identify risk factors associated with previous exposure.

Results

147 participants were enrolled in the study, provided blood samples, and completed a questionnaire. On ELISA testing, antibodies to the *Chlamydia* genus were detected in samples from 17 participants but further specific species-specific MIF testing did not detect *C. psittaci*-specific antibodies in any of these samples.

Conclusion

No serological evidence of past *C. psittaci* transmission from horses to humans was found in this study cohort. There are major challenges in using serological methods to determine the prevalence of *C. psittaci* exposure.

Keywords: Psittacosis, equine, zoonotic, serology

Introduction

Psittacosis is an under-diagnosed infection of zoonotic origin commonly associated with bird contact. This paper discusses the challenges of using serology for assessing the public health risk of psittacosis associated with horse-to-human transmission of *C. psittaci* in an Australian context.

Chlamydia psittaci is an obligate intracellular bacterium that causes the disease psittacosis in humans. Illness most commonly occurs following direct or indirect contact with infected birds.[1–4] Recent reports of purported equine-to-human transmission have highlighted a new potential transmission pathway which is not yet well characterised.[5–7]

The suspicion of equine-to-human transmission of *C. psittaci* first emerged in Australia in 2014 following an outbreak of respiratory illness associated with exposure to *C. psittaci*-infected equine foetal membranes at an Australian veterinary school.[5,7,8] In this cluster, all three symptomatic individuals who had serological testing performed were found to have *Chlamydia* genus antibodies detected on enzyme immunoassay (EIA) and *C. psittaci*-specific antibodies on microimmunofluorescence (MIF), although seroconversion (fourfold increase in immunoglobulin G titre, IgG) was not observed.[5] In 2016, investigation into reports of human respiratory illness, in close contacts of *C. psittaci*-infected horses (equine chlamydiosis) or of equine reproductive products, also suggested equine-to-human transmission, although psittacosis was not laboratory-confirmed on polymerase chain reaction (PCR) or MIF testing.[6] During a second outbreak of respiratory illness related to equine chlamydiosis at the initial veterinary school in 2017, one case of psittacosis was confirmed through PCR, with a further three probable cases based on serology (positive IgG).[9]

To inform a risk assessment of the zoonotic potential of equine chlamydiosis, we aimed to study the seroprevalence of antibodies to *C. psittaci* amongst an occupational cohort with close equine contact prior to the start of the 2017 foaling season. Although the study was at first designed as a longitudinal study with follow-up serology planned for the cohort following the end of the foaling season, the longitudinal study was not continued due to issues with the serological testing. This report describes the challenges encountered in the use of serological methods to determine the baseline prevalence of *C. psittaci* antibodies among historically exposed people.

Methods

Study concept

Individuals whose occupation involved close equine contact were tested for the presence of antibodies to *C. psittaci*. Participants included veterinarians, veterinary nurses and stud farm workers. Testing was planned to occur at two time points in 2017: before the foaling season (June 2017), to determine the baseline prevalence of antibodies to *C. psittaci;* and at the end of the foaling season (November 2017), so that the period prevalence of *C. psittaci* infection (i.e. seroconversion) could be calculated. It was expected that exposure to *C. psittaci* could occur during the foaling season, through contact with infected equine reproductive products and with sick foals, in the area that had previously had large confirmed seasonal outbreaks of equine chlamydiosis. Thus, a higher prevalence of antibodies amongst the cohort at the end of foaling season would indicate the extent of exposure during this time period. A questionnaire related to occupational risk factors was designed for administration contemporaneously with the serosurvey to identify risk factors associated with infection (Supplemental material 1).[i]

To determine the incidence of human psittacosis cases during the 2017 foaling season, any participants experiencing clinical symptoms compatible with psittacosis were to have a throat swab collected for PCR testing and complete a separate questionnaire detailing their symptom profile, exposures and any treatment received. Results from any PCR testing of symptomatic individuals could then be used for genomic comparison with equine specimens.

Advice from a One Health Equine Chlamydiosis Expert Advisory Group with representation from veterinarians, primary industry, microbiologists and public health specialists was obtained during the design of the study and throughout the duration of the project.

Ethics approval for the study was obtained from the Australian National University Human Research Ethics Committee (2017/320) and the Hunter New England Health Human Research Ethics Committee (17/05/17/4.05).

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Serological testing

All blood samples were screened for *Chlamydia* genus at the same pathology provider using the Medac Chlamydia IgG recombinant enzyme-linked immune assay rELISA (Medac, Wedel, Germany). IgG-positive samples (titre > 1:100) were then sent to a reference laboratory for microimmunofluorescence (MIF) species-specific testing using SeroFIA™ *C. psittaci* (Savyon Diagnostics Ltd, Ashdod, Israel). Detection of species-specific IgG antibodies to *C. psittaci* with titres ≥ 1:128 on MIF testing were considered evidence of current or previous infection with *C. psittaci*.

Questionnaire

A questionnaire collecting information on general demographics, occupational and non-occupational risk factors, and clinical symptoms related to psittacosis, was administered at the time of blood collection (Supplemental material 1)[ii].

Case definition

Definitions of confirmed and probable psittacosis used in this study were based on the Australian Department of Health national notifiable diseases psittacosis case definition, with the addition that contact with horses or with equine reproductive loss products represented epidemiological evidence of exposure.[10]

Results

In June 2017, 147 participants were enrolled into the study, provided blood samples, and completed the pre-foaling season questionnaire. The median age of participants was 32.8 years (range 19 to 63 years) and 106 (72.1%) were female. The majority of participants (n = 142, 96.6%) had worked with broodmares previously, with a median of ten previous foaling seasons (range 1–45).

Within the cohort of participants, the majority worked on equine stud farms (n = 101, 68.7%); 17.7% (n = 26) were veterinarians; 12.2% (n = 18) were veterinary nurses; and two (1.4%) were laboratory scientists involved in handling equine reproductive loss and post mortem specimens.

Fifty-nine participants (40.1%) reported, by questionnaire response, that they had previously been exposed to PCR-confirmed *C. psittaci*-infected horses or equine material during the 2015 and/or 2016 foaling seasons. Three self-reported a previous diagnosis of psittacosis based on positive serology, one during the 2015 and two during the 2016 foaling season; historical serology results were not available for review. A further two participants self-reported a clinician-suspected diagnosis of psittacosis based on clinical symptoms during the 2016 foaling season.

All samples that had a positive IgG on rELISA were sent for MIF testing (n=17). Of these 17 samples, no *C. psittaci*-specific antibodies were detected with MIF testing (Figure 1); six (35.3%) had positive antibodies to other chlamydial species (*C. pneumoniae* or *C. trachomatis*); and the other eleven samples (64.7%) did not have specific antibodies to any chlamydial species (Table 1).

During the 2017 foaling season no participants reported experiencing symptoms compatible with psittacosis, so no swabs were collected for PCR testing.

Absence of specific IgG antibodies to *C. psittaci* in the samples that underwent MIF testing raised concerns about the validity of the test, and the suspicion of false negative results. Given the concerns that serology did not accurately reflect the baseline prevalence of *C. psittaci* amongst the cohort, the initial screening results were discussed by the Equine Chlamydiosis Expert Advisory Group; the consensus was to not proceed with a second round of serological testing. This decision was made based on the consideration that if serological tests did not accurately reflect infection status it would lead

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Table 1: rELISA and MIF testing results for study participants and number of participants reporting previous history of psittacosis

	rELISA IgG negative (n)	rELISA IgG equivocal (n)	rELISA IgG positive (n)
Total number	**97**	**33**	**17**
History of psittacosis[a]			
Clinician diagnosed	1	1	-
Laboratory supported	-	2	1
MIF			
C. psittaci	n/a	n/a	0
Other	n/a	n/a	6
Negative	n/a	n/a	11

a Self-reported

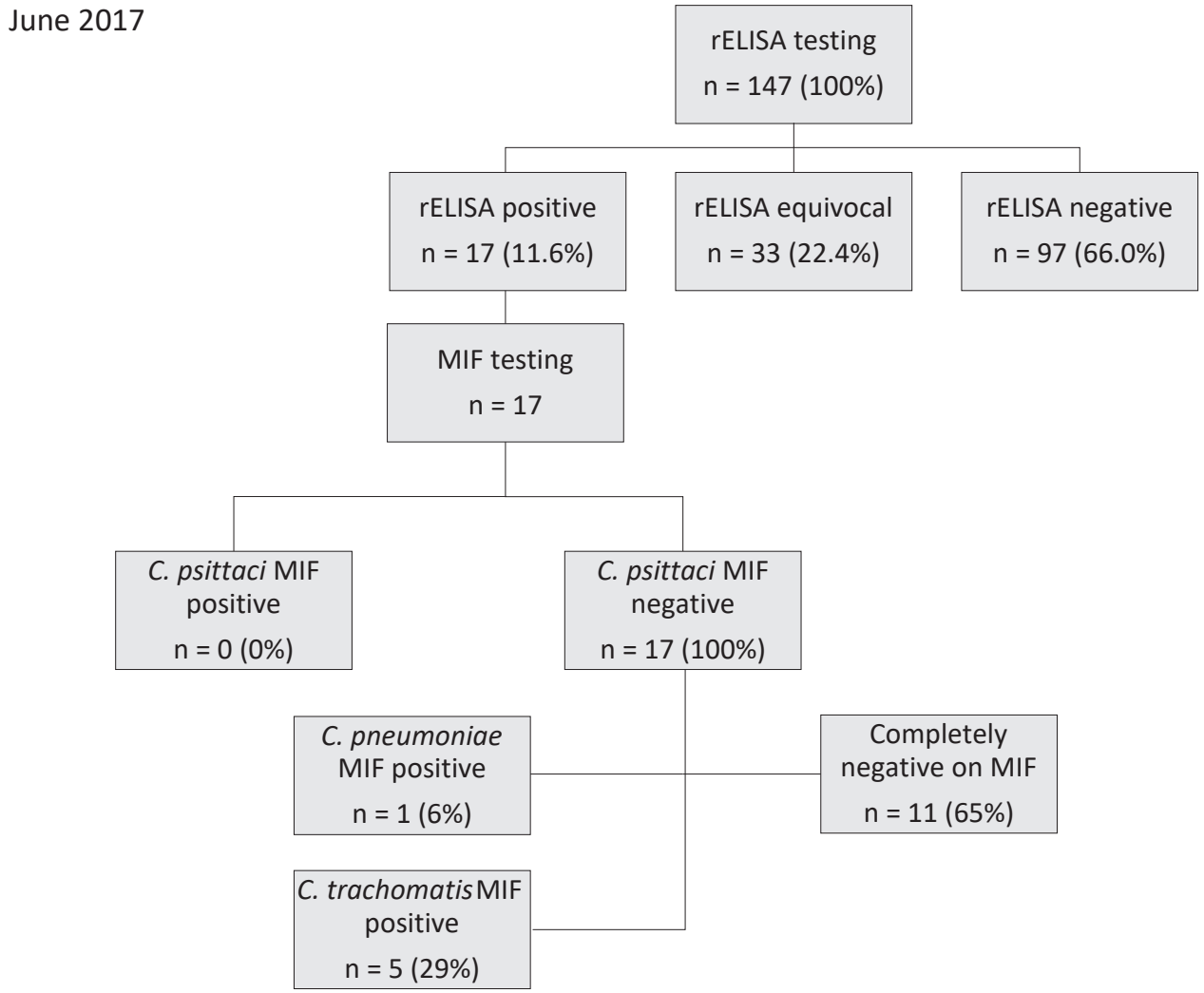
to case misclassification and make meaningful interpretation of the transmission risk difficult. Should the results from a second round of testing also return as negative, there would be a lack of confidence in concluding that the risk of transmission from horses to humans was actually negligible. An additional factor influencing this decision was the low prevalence of equine abortions associated with *C. psittaci* during the specific study year (2017), and thus likely lower levels of exposure during that foaling season.

Discussion

The absence of detectable *C. psittaci*-specific antibodies with MIF testing was surprising. It was expected that at least some individuals, particularly those who had reported a previous diagnosis of psittacosis, would have detectable antibodies. Given *C. trachomatis*, *C. pneumoniae*, and *C. psittaci* are the most common species causing human infections, with infection by other species being rare, the 11 samples with no species-specific antibodies detected on MIF could have been false positives on rELISA. Alternatively, they could represent the presence of species-specific antibodies, potentially to *C. psittaci*, that were not detected by MIF either due to poor sensitivity or low levels of antibody. Whilst it is recognised that the MIF assay is susceptible to false negative results, we expected that this test would have some capacity to detect previous exposure in this cohort given the large sample size and detection of antibodies reported in other studies involving smaller case numbers, such as the veterinary school cluster. MIF testing has also been used in other *C. psittaci* seroprevalence studies, and whilst the true burden of disease may remain underestimated due to possible false negative results, some measure of prevalence was able to be assessed in those studies.[11,12]

Our study results could indicate that participants had no previous infection with *C. psittaci*, although if *C. psittaci* is indeed zoonotic, this would be unlikely given that a high proportion of participants (40.1%) reported direct exposure to *C. psittaci*-infected horses or equine products during the previous foaling season. Furthermore, of the five participants who reported a previous diagnosis of psittacosis (either self-reported laboratory-confirmed or clinician diagnosed), only one had detectable IgG on rELISA and was negative on MIF. This raised the suspicion of false negative serology results.

Figure 1: Flowchart of the testing pathway and results of *Chlamydia* genus-specific rELISA testing and species-specific MIF testing



The type of *C. psittaci* antigen used in MIF testing can affect the performance of the test.[13] Genomic analysis of *C. psittaci* isolated from equine reproductive loss specimens from NSW revealed that the strains were clonal and belonged to the 6BC parrot-associated strain.[14] Differences between the *C. psittaci* elementary body antigen in the 6BC strain circulating among Australian psittacines and the elementary body antigen used in commercial diagnostic kits could result in reduced antibody affinity, leading to false negative results on MIF. However, without an Australian psittacine-specific antigen available, it is impossible to compare samples and test this hypothesis. Differential antibody affinity has been observed in other studies. Wong *et al.* reported some variability in antibody titres to different strains of *C. psittaci* antigen tested.[15] They also found that 30% of clinically-diagnosed cases of psittacosis were negative for *C. psittaci*-specific antibodies with MIF testing.[15] Other studies have also reported presumed false negative results with MIF testing. Verminnen *et al.* found no evidence of *C. psittaci*-specific antibodies using MIF testing in three study participants despite all three being positive for chlamydia-genus antibodies on ELISA and PCR-positive on nasal and pharyngeal swabs.[16] Dickx *et al.* also reported a study participant who was *C. psittaci* PCR and culture positive but who remained seronegative on MIF testing.[17]

It is possible that antibody levels in previously-exposed individuals were below the level of

detection using MIF testing. Early antibiotic treatment during symptomatic infection has been reported to inhibit antibody development, leading to false negative serological results.[18,19] It has also been postulated that passage through a non-avian host (such as horses) could decrease the virulence of *C. psittaci*, which in turn could lead to a reduced host-antibody response in humans.[2,20] However, the severe disease requiring hospitalisation reported in some of the Australian veterinary school outbreak cases does not support the theory of attenuated virulence.[5,9] Antibody levels may have also waned over time to below the level of detection. There is little information available about the duration of the humoral antibody response to *C. psittaci*. A previous study investigating the persistence of chlamydial antibodies after an outbreak of psittacosis in turkey industry workers found that 73% of cases had no detectable antibody on complement fixation testing nine months following infection.[21] In the current study, blood samples were taken seven months after the previous foaling season ended, so antibodies may have declined over this time. Finally, given *C. psittaci* is an intracellular pathogen that generates a predominantly cell-mediated immune response, antibodies may not be an appropriate marker of infection.[2]

As a consequence of the lack of a gold standard method for detecting *C. psittaci* infection in humans, it is impossible to determine whether the results of this study truly reflect an absence of *C. psittaci*-specific antibody or instead indicate an inability of the test to detect antibodies.[13,22] Furthermore, without comparison to a gold standard test, the sensitivity and specificity of MIF cannot be evaluated, further clouding the interpretation of results. Thus, whether due to low levels of circulating antibodies or low sensitivity of MIF testing, serology may not accurately reflect a person's history of infection with *C. psittaci*.

The difficulties with serological testing experienced in this study are not unique. Several studies have reported the absence of positive MIF serology in cases of clinically and epidemiologically suspected psittacosis, highlighting the issue of potential false negative results.[5,15,16,23–25] In the absence of information on test sensitivity, the degree to which these tests fail to represent previous exposure remains unknown. Serology may still be beneficial in acute infection in combination with polymerase chain reaction (PCR) testing; however, the baseline results from this study suggest it is not a reliable method for determining the prevalence of previous exposure.

Whilst the use of PCR in diagnosing acute infection with *C. psittaci* is preferable, it also has its challenges. Several studies have shown discordance between PCR and serology results; there have been reported difficulties getting adequate clinical samples for testing; and PCR is also susceptible to false negative results.[16,17,22,26] For example, an investigation into an avian-related outbreak of psittacosis in a veterinary teaching hospital in the Netherlands found that in 4 of the 7 serologically-confirmed cases, PCR on throat swab samples was negative.[26] Even the PCR results in samples taken from the same person at different sites (throat, urine or sputum) can differ, and the sensitivity of PCR declines quickly with time after infection.[16,22,27] The rapid decline in PCR positivity following acute infection limits its use in determining the baseline prevalence of previous exposure.[22] Despite this, some studies have used PCR screening to assess the prevalence of *C. psittaci* infection in high-exposure populations.[17,28] Whilst the sensitivity of PCR in this setting has not been determined, it would offer the additional benefits of providing information on subclinical infection and the spectrum of disease, as well as the ability to genomically link any human cases with equine specimens.

The issues with current *C. psittaci* serological testing methods limit their utility in epidemiological studies, including exploring alternate exposure pathways such as equine-to-human transmission. Improved testing methods would allow for better characterisation of the public health risk of *C. psittaci* transmission from horses to humans and help to gain a better understanding of the full spectrum of disease.

Conclusion

We found no serological evidence of previous exposure to *C. psittaci* in our occupational study cohort. A second round of pathology testing was not conducted due to concerns over the reliability of serology to accurately reflect exposure to *C. psittaci*. We would not recommend using MIF to assess the point prevalence of *C. psittaci* exposure.

A greater understanding of the transmission of *C. psittaci* between horses and humans has potential public health implications. Efforts to assess this risk will continue to be limited unless improved testing methods can be developed.

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