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An outbreak of psittacosis at a veterinary school demonstrating a novel source of infection

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ABSTRACT

In November 2014, New South Wales Health was notified of a cluster of respiratory illness in a veterinary school. Active case finding identified another case at a local equine stud. All cases had exposure to the equine fetal membranes of Mare A. This tissue subsequently tested positive for *Chlamydia psittaci* using quantitative real-time polymerase chain reaction. We conducted a cohort study of the university and stud farm staff to determine risk factors for disease. Nine people were exposed to the fetal membranes of Mare A. Of these, five cases of psittacosis were identified. Two required hospital admission. Contact with birds was not associated with illness (RR = 0.5, 95% CI = 0.09–2.73). People who had direct contact with the abnormal fetal membranes were more likely to develop disease (RR = 11.77, 95% CI = 1.02–∞). The emergence of an association between horse exposure and *C. psittaci* infection has important implications for the prevention and control of psittacosis.

Article summary line: Investigation of an outbreak of psittacosis in a rural veterinary school demonstrates novel source of infection for psittacosis through exposure to abnormal equine fetal membranes.

1. Introduction

Psittacosis is a systemic infectious disease caused by *Chlamydia psittaci*. It is characterised by fever, malaise, myalgias and atypical pneumonia [1]. Complications include myocarditis, endocarditis, hepatitis, reactive arthritis, and neurological abnormalities [2]. Birds are the major zoonotic reservoir [3]. However, outbreaks of psittacosis have occurred in the absence of direct bird exposure. Epidemiological studies have demonstrated associations time spent outdoors and the suggested mechanism of infection is through aerosolisation of infectious particles shed by birds through the process of lawn mowing [5,6]. There is also limited evidence of person to person spread [7].

Previous studies have demonstrated the presence of *C. psittaci* infection in horses [8,9], however, to our knowledge transmission to humans has not previously been demonstrated [4]. Limited studies suggest horses are only occasional hosts of *C. psittaci*, and infections in horses can cause respiratory disease and fetal abortion [8–10]. We describe an outbreak of probable psittacosis within a veterinary school linked to exposure to infected equine fetal membranes, in which we aimed to establish the likely route of exposure.

2. Background

On 21 November 2014, the Department of Primary Industries, the government department overseeing agriculture in New South Wales (NSW), reported four cases of respiratory illness among staff and students at a veterinary school to Health Protection New South Wales (NSW). The veterinary school is located in Wagga Wagga, a city with a population of approximately 63,000 persons, located in rural Australia. The affected staff worked in the veterinary reproduction unit where students were undertaking a rotation at the time. Active case finding identified a further case of atypical pneumonia in a human at a local equine stud, where one of the students worked. Through this outlier, we established a common exposure to the equine fetal membranes of Mare A among all the cases, generating the hypothesis that the

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membranes of Mare A were the source of the outbreak. The fetal membranes subsequently tested positive for *C. psittaci* using quantitative real-time polymerase chain reaction (qPCR). An outbreak investigation team, coordinated by Health Protection NSW, was set up. Health Protection NSW has statutory responsibility for disease control and surveillance in NSW.

3. Methods

3.1. Case definition and identification

We defined a suspected case as a person who reported exposure to the fetal membranes of Mare A and who had clinical features of community acquired pneumonia OR at least two symptoms of fever, headache, myalgia, dry cough or dyspnoea in Wagga Wagga, between 1 November and 14 December 2014. In addition to the features above, we required a probable cause to have a single high IgG titer (>32) to *C. psittaci* demonstrated by micro-immunofluorescence (MIF) and a confirmed case to have a fourfold rise in antibody titer to *C. psittaci* by MIF or detection of *C. psittaci* by nucleic acid testing.

Active case finding was conducted at the veterinary school through an online communication system for students and an email and newsletter for staff. Additional cases were also identified by interviewing cases.

3.2. Epidemiological investigation

We conducted a site visit to the equine stud and veterinary school on 5 March 2015. We interviewed all persons exposed to the equine fetal membranes of Mare A using a standard questionnaire designed for the outbreak. We collected information on demographics, clinical history, laboratory investigations, and potential exposures. Degree of exposure to the membranes was further delineated into the following non-exclusive categories: 1) attendance at the examination of the membranes, 2) scrubbing of contaminated floors, 3) direct contact with bagged membranes, 4) direct contact with exposed membranes, and 5) manipulation of membranes (i.e. inverting the membranes for examination). The use of personal protective equipment (PPE) was also examined.

We compared proportions by case status using the Wilcoxon rank-sum and Fisher's exact tests. We calculated relative risks (RR) and 95% confidence intervals (95% CI) to quantify the association between exposure variables and illness. We used exact logistic regression to calculate an odds ratio (OR) approximating the RR, when RR was unable to be calculated. Data analysis was performed using Stata IC 13.

3.3. Laboratory investigation

Respective treating clinicians obtained acute and convalescent serological samples from three of the five cases. Sera were tested for *Chlamydia* sp. antibodies. Using enzyme immunoassay (EIA) and further differentiated by species (*Chlamydia psittaci*, *Chlamydia pneumoniae* and *Chlamydia trachomatis*) using microimmunofluorescence (MIF). For the two hospitalised cases, serology for Q fever, leptospirosis, brucellosis, mycoplasma, toxoplasma, Ross River virus, and urinary legionella and pneumococcal antigens were collected to exclude other likely diagnoses. A serology sample was also obtained from the mare and tested for *Chlamydia* sp. EIA. The equine fetal membranes were stored at −20 °C in a freezer at the veterinary school (for teaching purposes). We investigated the membranes with qPCR and examined histologically. To address the potential of environmental contamination, we swabbed the internal aspect of the membranes – this method has been validated in subsequent investigations of aborted fetuses, showing concordance of internally sampled membranes with sampling from the fetus (Gabor M, personal communication, 17 October 2016). qPCR was performed targeting genes specific for *C. psittaci*, *C. trachomatis*, *C. pneumoniae* and *C. abortus*, as well as *Coxiella burnetii*, *Rickettsia* spp. and Hendra virus. DNA was extracted from 10 to 20 mg of tissue using the Wizard® Genomic DNA Purification Kit (Promega, WI USA) according to the manufacturer's instructions. Chlamydial qPCR was performed using a Corbett real-time platform with genus-specific [11,12] and species-specific primers for *C. psittaci* [13–16]. qPCR tests for *C. pneumoniae* and *C. trachomatis* DNA were performed in parallel, using primers based on published species-specific sequences to exclude inadvertent species cross-reactions [14,15]. For histology, formalin fixed tissues were embedded in paraffin, sectioned (5 μm) and stained with hematoxylin and eosin.

4. Results

4.1. Descriptive epidemiology

A total of nine people were exposed to the fetal membranes of Mare A. Mare A, normally kept at a farm in Goulburn, NSW was transferred to a stud farm in Wagga Wagga three weeks before foaling. She foaled on 5 November 2014 with two stud staff in attendance. A third stud staff member examined the membranes and stored them in a porous plastic feed bag later in the morning. The membranes were grossly abnormal: the chorionic surface of the fetal membranes displayed a diffuse dark red to black discolouration. (See Fig. 1). The foal died one week later – the cause of death was not further investigated by stud staff.

The membranes were taken to the university by one of the staff members at the stud, who was also a student at the university, and examined by two academic staff and three students for teaching purposes. The examination involved inverting the membranes. The next day, a technical staff member from the university transferred the bagged membranes into a watertight bag and stored it in the freezer.

From this cohort of nine, a total of five cases of psittacosis (three probable, two suspected) were identified; an attack rate of 56%. The clinical features of the five cases are summarised in Table 1. All cases reported fever, fatigue, headache and clinical signs of pneumonia as identified by medical practitioners. Four of the five cases received chest X-rays and all had evidence of lobar consolidation. The onset dates of illness ranged from 9 November – 17 November 2014. (Fig. 2) Overlapping periods of exposure, calculated from onset dates based on an incubation period between 5 and 14 days, indicated a likely point-source exposure between 3 and 5 November, consistent with the known date of exposure to the fetal membrane of Mare A on 5 November. Active case-finding at the university did not identify any cases of atypical pneumonia not linked to the membranes over the same period.

Two cases were hospitalised and three cases were treated by primary care physicians. The case descriptions of the hospitalised cases below:

University staff member A was a 25 year old female admitted on 19 November 2015 with fever (40.1 °C), headache, back pain, myalgia and

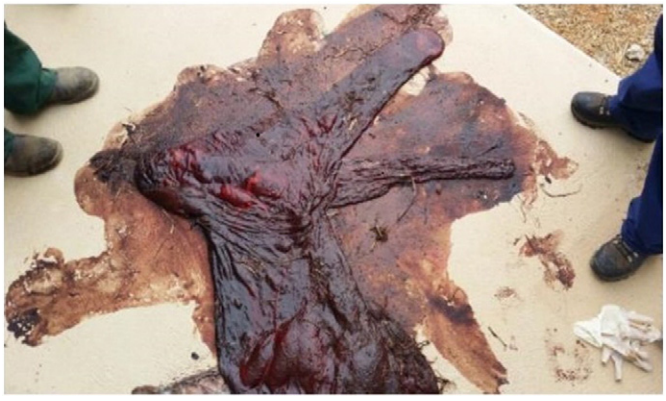

Fig. 1. Infected equine fetal membranes from Mare A,Wagga Wagga, 5 November 2014.