New insights into chlamydial zoonoses

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Chlamydiae are obligate intracellular bacterial pathogens of humans. Infections in animals are also widespread with some species, such as *Chlamydia psittaci*, long recognised as a serious threat to human health. Critical to the public health response of any zoonotic disease outbreaks is reliable and up-to-date information on the epidemiology of the target pathogen. Aided by advances in the use of quantitative PCR, molecular typing and culture-independent genomic studies, significant recent work has highlighted an expanded diversity and host range of chlamydial pathogens in animals. New and unexpected cases of chlamydial zoonoses have now been recently documented in Australia and elsewhere, emphasising the importance of multi-disciplinary 'One Health' collaboration and the use of standardised methods to detect and characterise chlamydial pathogens in humans and animals.

A brief history of chlamydial zoonosis

The first recognition of the zoonotic potential of chlamydial infections predates the actual description of the bacteria[1]. In 1879, Jacob Ritter described an epidemic of fatal respiratory disease in humans associated with contact with caged parrots and finches. At the time, the aetiological agent of this disease, later coined psittacosis, was unclear although it was suspected that it was of viral origin. Interest in the disease re-emerged in 1929–1930, with epidemics of human disease reported in Europe and the Americas, again linked to infected and imported parrots. The global attention generated from these outbreaks prompted several years of fruitful research, including important studies by Australia's Sir Frank Macfarlane-Burnet[2, 3], ultimately leading to the description and characterisation of a bacterium, *Chlamydia psittaci*, with a complex biphasic developmental cycle[1]. Since this time, *C. psittaci* has been considered the classical chlamydial zoonotic pathogen, with zoonotic transmission and acute, serious disease (in the form of atypical pneumonia) resulting from direct contact with infected birds or their contaminated excreta[4]. Historically, most of the attention is rightly placed on the pathogenic potential of *C. psittaci*; however, isolated cases of the zoonotic transmission of closely related *Chlamydia abortus* from sheep have also been documented, the latter linked to subsequent abortion in pregnant women that are exposed to the secretions of *C. abortus* infected ewes[5].

Growing recognition of the diversity of chlamydial infections in animals

Bacterial adaptation to an obligate intracellular niche would typically imply genetic conservation and a restricted host range. As we have learned more about the diversity of taxa within the phylum Chlamydiae, new surprises continue to emerge. Recent years have seen the proposal and description of several new order and family level linages of chlamydiae[6]. Nevertheless, most attention remains on the genus *Chlamydia* since it comprises a number of important human and animal pathogens. Since the relatively recent (re-) classification of chlamydial species into a single genus[7], a plethora of new species (14 total species in the genus) have now been proposed or formally classified[6] beyond the 11 that were initially included. The documented host range of some of the most well described of these chlamydial species in the genus *Chlamydia* is shown in Figure 1. Supported by the use of molecular tools such as family- and genus-specific qPCR assays targeting conserved chlamydial sequences[8] of widely conserved eubacterial ribosomal rRNA genes, these discoveries have primarily come on the back of veterinary investigations into previously recognised warm-blooded

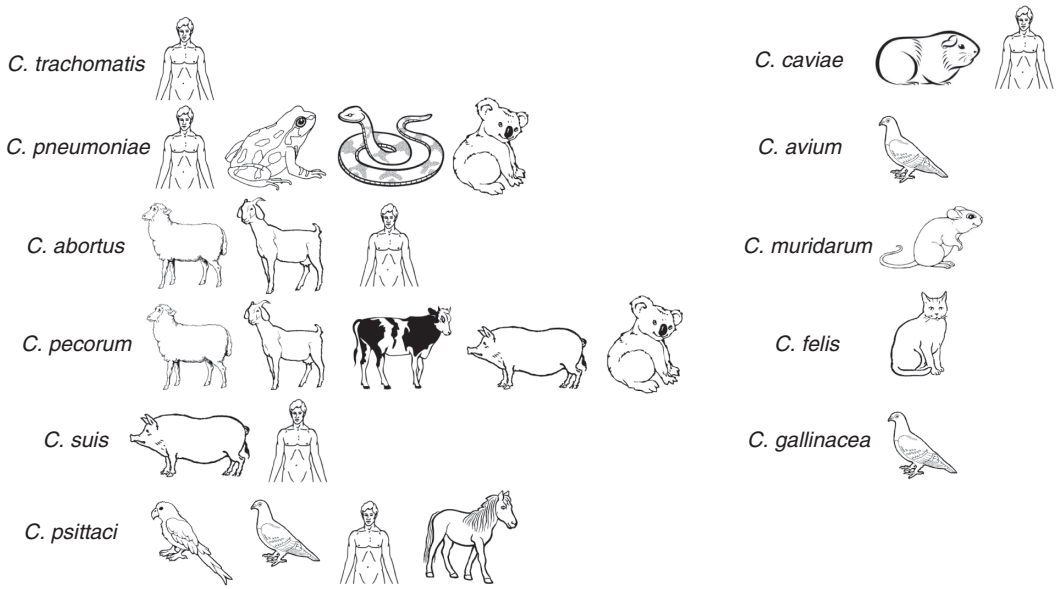

Figure 1. Diversity of different chlamydial pathogens in the genus *Chlamydia* and their documented hosts. Recently described reptilian chlamydial species, *C. serpentis, C. poikilothermis* and *C. buteonis* are not shown.

hosts of chlamydial infections as well as new hosts such as snakes[9–12]. While the pathogenic potential of many of these newly described chlamydial pathogens remains unclear, their discovery has highlighted how little is still yet known about the diversity and epidemiology of chlamydiae.

Advances in molecular tools to study chlamydial epidemiology and zoonotic events

A number of technical innovations over the past 20 years in the chlamydial research community have paved the way for a greater understanding of chlamydial epidemiology and the documentation of infection spill-over events from recognised and emerging chlamydial zoonotic agents. The first of these is the shift from laborious culture-based methods for detecting chlamydial infections to the use of conventional and quantitative PCR-based methods that detect the presence of chlamydial DNA[13]. Coupled with the use of the aforementioned broad-range order and family-specific primers[8] to 'cast a wide net' in the screening of clinical specimens, these approaches have revolutionised the sensitive detection for chlamydial pathogens in human and animal samples.

An interesting example of the use of these approaches to uncover an unexpected potential for chlamydial zoonoses comes from studies in Belgian pig farms and slaughterhouses[14–16], including one study where researchers have documented the presence of *Chlamydia suis*, a ubiquitous pig pathogen, in the mucosal swab samples (conjunctival and rectal) collected from farmers and slaughterhouse workers[15]. This discovery is of particular concern given that *C. suis* harbours the only known naturally occurring antibiotic resistance cassette in the *Chlamydiaceae*, raising fears over the potential to transfer this genetic element to the closely related human chlamydial pathogen, *Chlamydia trachomatis*[17,18]. A potentially legitimate criticism of the use of DNA-based methods for detection of chlamydial pathogens in new hosts is that the detection simply reflects contamination or exposure and not genuine infection that might lead to disease and/or subsequent chlamydial shedding. To rebut this criticism, the Belgian team also detected the presence of species-specific antibodies in the human subjects, providing stronger evidence for the zoonotic potential for *C. suis*[16]. The challenge in doing so for other chlamydial species suspected of zoonotic transfer is an almost complete lack of serological tools for measuring specific human host responses to the growing and diverse range of chlamydial pathogens infecting animals. In an exciting advance to the field, this may become easier in the future with the recent development of highly specific peptide microarray assays for detecting *Chlamydia*-species specific antibodies in human and animal sera[19]. Even though it is only pilot data, it is interesting to note that studies of small selections of samples from livestock with these assays uncovered specific antibody responses to a diverse range of chlamydial pathogens, potentially suggesting that the previously postulated host barriers for most species in the genus *Chlamydia* do not actually exist[19].

Another important technical advance has been the development and application of multi-locus sequence typing schemes (MLST) to study the fine-detailed molecular epidemiology of chlamydial pathogens. This standardised approach utilises DNA sequences of conserved 'house-keeping' genes that, when combined, create a unique profile for each genetically distinct strain sequenced[20]. Schemes have now been developed for most species in the genus *Chlamydia*, creating opportunities to interrogate strains of the

same chlamydial species from different hosts to gain insight into their relationship and the potential for cross-host transmission, as will be discussed below. These approaches have become even more powerful when coupled with the use of culture-independent genome sequencing technologies to obtain the full genome sequence from strains in clinical samples, opening the possibilities for interrogating 'field-relevant' strains[21].

'One Health' investigations document new cases of chlamydial zoonoses in Australia and the rest of the world

While technological advances have opened up new opportunities to perform surveillance of potential chlamydial zoonotic events, they are only useful if employed as a part of multidisciplinary teams of experts including doctors, veterinarians, public health staff and microbiologists.

The best example of a recent 'One Health' partnership to conduct surveillance of zoonotic chlamydial infections comes from The Netherlands. In this report, *Chlamydia caviae*, a guinea pig pathogen was found to be the causative agent of severe pneumonia in three unrelated human cases[22]. In all cases, the humans had developed symptoms after being exposed to ill guinea pigs. Courtesy of an agreement between veterinary and human diagnostic laboratories to use the same molecular detection and typing methods[23], the authorities were able to confirm the suspected transmission by showing that the strains from the human subject and diseased guinea pig were identical in at least one case.

Closer to home, a team of human and veterinary clinicians and researchers have provided important new insight into the zoonotic potential of the avian and zoonotic pathogen, *C. psittaci.* A cluster of cases of probable psittacosis were detected amongst veterinary students and staff at regional university veterinary school in New South Wales[24]. The ensuing public health investigation revealed shared contact with the infected placental membranes of a mare that had delivered a foal that died prematurely. Fine-detailed molecular analysis by our team, including application of a species-specific *C. psittaci* MLST scheme, revealed that the sample contained a *C. psittaci* strain belonging to the avian 6BC clade[25], responsible for the global epidemics in the 1930s and found in Australian native parrots[26]. The genetic relationships of the newly detected equine *C. psittaci* strains to strains from other hosts is illustrated in Figure 2. This discovery provided the first evidence of a potential mammal to mammal transmission of *C. psittaci* and revealed a new zoonotic risk by this chlamydial agent. Subsequent studies by our team have further revealed that: (1) avian *C. psittaci*

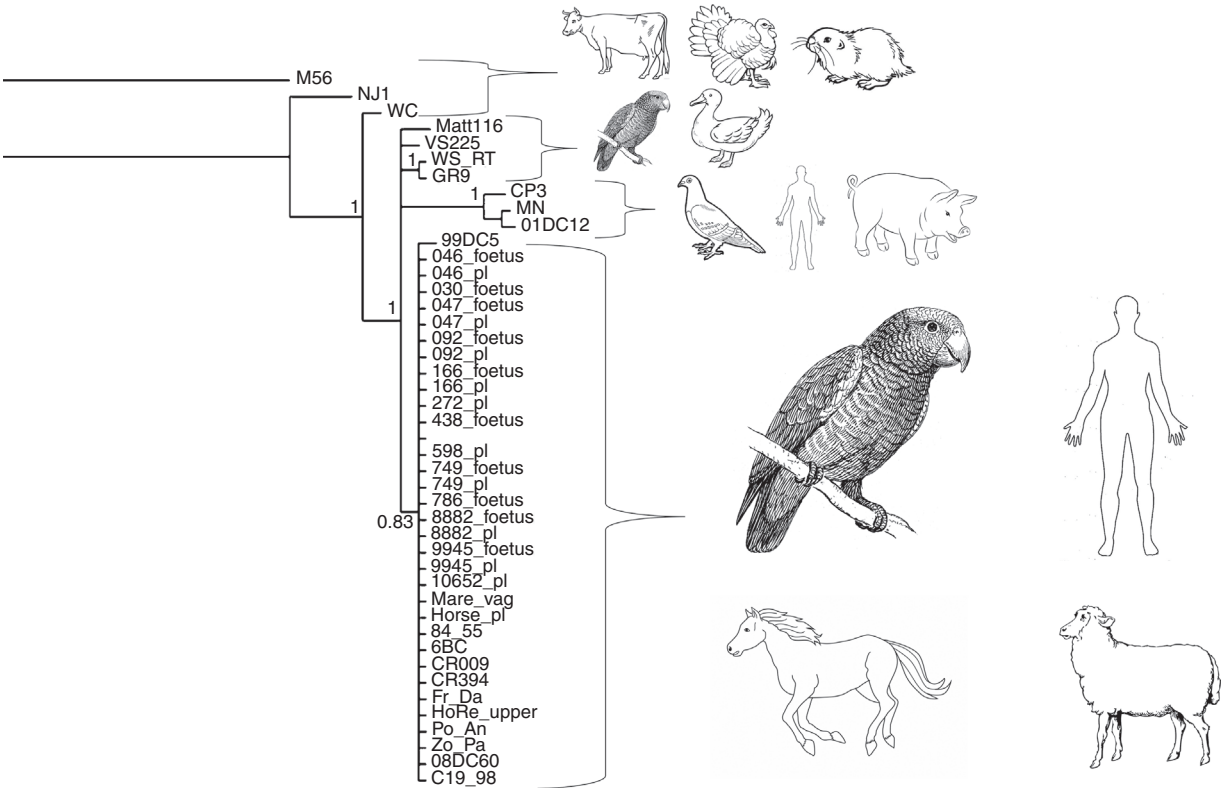

Figure 2. A mid-point rooted Bayesian phylogenetic tree using the alignment of the concatenated MLST fragment sequences from 44 *C. psittaci* strains reveals the genetic relationships between strains detected within and between different hosts. The main clade of virtually identical strains contains *C. psittaci* isolates derived primarily from Australian parrots, humans and horses. Bootstrap values are indicated on the nodes. Hosts for the strains in each clade are displayed. Modified from Jenkins *et al.*[27].

infections are a more common cause of reproductive loss in mares than previously thought[27]; (2) the subsequent occupational risk to veterinarians may be significant; and (3) other *C. psittaci* strains beyond those found in parrots may also infect horses and, hence, potentially pose a risk to human health[28].

Future directions in understanding chlamydial zoonoses

This review has highlighted a growing awareness of the zoonotic potential of a broad range of chlamydial pathogens both in Australia and abroad. This new information comes largely on the back of improvements in the surveillance of chlamydial pathogens with new technologies increasing our ability to detect and monitor these intracellular bacteria in different hosts. An awareness of the host range and pathogenic potential of animal chlamydiae in humans is important information that can be used to guide surveillance efforts by public health authorities.

As the next step in efforts to predict and minimise the risk of chlamydial zoonoses, one area of research that needs significant more work is in understanding what factors influence chlamydial spill-over events between animals and humans. For example, in the case of equine *C. psittaci* infections, what specific factors influence transmission of the pathogen in birds and, hence, present a risk of infection to humans? Prior to the detection of *C. psittaci* infections in horses, have infection spill-over always occurred historically or are there specific changes in the local bird ecology that increase the risk of *C. psittaci* shedding in the environment? One Health partnerships between human and veterinary stakeholders will continue to be at the forefront of efforts to answer these questions for chlamydiae and other zoonotic agents.

Conflicts of interest

The authors declare no conflicts of interest.

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