# Persistent or long-term coronavirus infection in Australian bats

*Craig Smith*

University of Queensland
Brisbane, Qld, Australia
Email: craig.smith@daf.qld.gov.au

When the World Health Organization declared the end of the global outbreak of severe acute respiratory syndrome (SARS) on the 5 July 2003, more than 8000 cases with over 800 fatalities had been reported in 32 countries worldwide and financial costs to the global economy were close to $US40 billion[1,2]. Coronaviruses were identified as being responsible for the outbreaks of both SARS and Middle East respiratory syndrome (MERS, the latter in 2013). Subsequently, bats (order Chiroptera) were identified as the natural hosts for a large number of novel and genetically diverse coronaviruses, including the likely ancestors to SARS-like and MERS-like coronaviruses[3–8].

Coronaviruses, of the order *Nidovirales*, family *Coronaviridae*, are the largest known non-segmented, single stranded, positive sense RNA viruses (28 to 32 kb). They have large projections protruding from the envelope that are formed by trimers of the spike protein and when viewed by electron microscopy form the characteristic 'crown' that gave rise to the family's name. Coronaviruses can cause a range of syndromes including respiratory and gastroenteric disease in humans and respiratory, gastroenteric, neurological and hepatic disease in animals, often with significant economic consequences. Respiratory and faecal-oral transmission are common but biological vectors are not known. Pigs, cats and domestic fowl may become persistently infected and shed virus from the enteric tract, many doing so for a lifetime[9–13].

## The ecology of bat coronaviruses around the world

Surveillance and identification of bat coronaviruses continues to occur around the world, most recently with the detection of SARS-like and MERS-like coronaviruses in bats in Korea, and the demonstration of genetically diverse clusters of bat coronaviruses

in the Atlantic Forest Biome, Brazil[8,14]. However, in spite of these investigations and the potential serious consequences of these high-profile pathogens, knowledge of their ecology is still limited. For example, it is still unknown how these coronaviruses are maintained, amplified or controlled by their chiropteran hosts[15].

Previous studies by Drexler *et al.*[15] identified two peaks of amplification of coronaviruses, characterised by increased virus concentration and increased detection rates, upon the formation of a colony of *Myotis myotis* in Germany and following parturition. It was hypothesised that the initial peak was probably due to the formation of a colony of sufficient size and density to allow the establishment of a viral transmission cycle in susceptible bats. The second peak, after parturition, was associated with the introduction of susceptible bats, newborn pups who had lost their perinatal protection but not yet mounted their own adaptive immunity[15]. In another attempt to better define the epidemiology of coronaviruses, Lau *et al.*[16] marked 511 Chinese horseshoe bats (*Rhinolophus* spp) from 11 sites and recaptured 113 (22%). From this study it was estimated that viral clearance occurred between two and 16 weeks after infection and suggested that coronaviruses in Chinese horseshoe bats caused an acute self-limiting infection associated with weight loss. It was also identified that the peak activity for coronaviruses was during spring, soon after hibernation, and that mating and feeding activity may have facilitated the spread of the virus within and between roosts.

## Persistent or long-term infection of Australian bat coronavirus

Subsequent to these ecological studies, we identified four putative novel coronaviruses (two *Alpha-* and two *Betacoronaviruses*) in seven species of Australian bats[17,18]. One of these species (*Myotis macropus*, Figure 1), had individuals infected with a putative novel *Alphacoronavirus* (detection of coronavirus RNA in faeces from bats enrolled in a mark-recapture study) over periods of up to 11 weeks, supporting the hypothesis for persistent or long-term infection as a method of maintaining coronaviruses in bats[17,19].

This period of infection (up to 11 weeks) was consistent with that observed by Lau *et al.*[16] of between two and 16 weeks. However, whereas Lau *et al.*[16] suggested that SARS-Rh-BatCoV caused an acute, self-limiting infection in individual Chinese horseshoe bats, our interpretation would be that the Australian *Alphacoronavirus* appears capable of a persistent or long-term infection of bats. Persistent infection has previously been suggested as

playing a role in the maintenance of coronaviruses in populations of bats, as it does for other coronaviruses, including feline coronaviruses (FECV) where it has been shown that naturally infected cats shed FECV intermittently for periods up to 10 months but some (~15%) become chronic shedders, doing so for years or a lifetime[12,13,20–22].

The apparent discrepancy between an acute infection observed by Lau *et al.*[16] and a persistent infection interpreted from this study's results requires clarification. It is possible that the discrepancy is real and there are true variations in patterns of infection for different species of coronaviruses and bats, or it could be that the limited rate of recapture of infected bats in the study by Lau *et al.*[16] precluded an accurate interpretation of infection. Whilst Lau *et al.*[16] made a significant effort in marking 511 bats, only 113 (22%) bats were recaptured and coronavirus was only ever detected in 63 of the 511 bats (12%). Of these 63 bats, shedding of coronavirus was detected in only one bat on more than one occasion (two weeks apart) and 10 bats which were detected shedding coronavirus at one sampling event were not detected shedding when recaptured (between 4 and 16 months later), providing an interpretation of an infectious period of between 2 and 16 weeks (4 months). Conversely, whilst only employing 52 marked bats, our study had a viral prevalence of 54% (28 bats) and a recapture rate of 81% (42 bats). The weekly sampling events and the affinity of bats for their roost, provided a unique opportunity to frequently recapture marked individuals that were shedding coronavirus (Figure 2). This increased probability of recapturing bats allowed interpretation of the pattern of infection for our longitudinal study and reasonably suggested a persistent infection of coronaviruses in Australian bats[19].



Figure 1. A female *Myotis macropus* and her 2-week-old pup. This female had an implantable radio frequency identification transponder, more commonly known as a 'microchip', subcutaneously implanted on the dorsum during Week 2 of the mark-recapture study, when she was identified (by palpation of the abdomen) as being pregnant. She was recaptured on Week 4 and was again identified as being pregnant, on Week 5 she had given birth and the pup was attached. On Week 7 the pup was still attached and they were both photographed. When recaptured on Week 12 the pup was no longer attached and was assumed to have weaned, roosting separately with the other weaned pups that were observed in the colony[19]. Photograph courtesy of Steve Parish.

Previous studies suggested that physiological stress associated with pregnancy and lactation was a risk factor for increased seroprevalence of virus infections in bats[26,27]. Similarly, a correlation between the detection of coronaviruses in female bats associated with maternity colonies has also been established[28,29]. The colony used in our study had been selected for its ease of access and the high affinity of bats to the roost, providing a successful recapture rate. It was opportunistically and irregularly sampled over the previous year, with a coronavirus RNA detection prevalence of



Figure 2. A collapsible bat trap. The collapsible bat trap (*a*), commonly known as a harp trap was developed by Tidemann and Woodside[23] based on the original designs of Constantine[24] and Tuttle[25]. The trap is a common tool used for the capture of insectivorous bats and is best placed in the natural flight path of bats, including; roads, trails, streams and roost entrances. The trap is light and portable and can be set up in 5 minutes by a single person. The author removing captured bats from the bag of a harp trap (*b*).

between 30% (19–45%, 95%CI) one year prior to the commencement of the mark-recapture study, and 0% (0–15%, 95%CI) three months prior. Only during the first sampling event did we identify that the majority of female adults (88%) were pregnant and that the study site should be considered a maternity colony. In agreement with other studies[15,28,29], it appears that the site had an increased prevalence of coronavirus when used as a maternity colony (during the mark-recapture study and exactly one year prior), as opposed to other times (three months prior) when no coronavirus was detected and no pregnant females were observed. However, modelling the presence or absence of coronavirus (using logistic regression) did not show any association with the animal risk factors pregnancy or lactation status, and suggests that physiological or environmental stressors are not driving coronavirus infection in Australian bats[19].

Alternatively, migration of bats has been shown to play a role in the maintenance of viruses; immigration allows the maintenance of an infection through newly introduced susceptible individuals[30,31]. However, the population of bats used in our study appeared relatively closed with the population size remaining between 72 and 101 bats and apparent high fidelity to the roost site (assumed from the high recapture rate of marked bats, 81%). It is therefore unlikely that immigration of susceptible bats was responsible for the maintenance of the *Alphacoronavirus* in this relatively small and closed population. Throughout a three year study, Drexler *et al.*[15] observed that strong and specific amplification of RNA viruses, including coronaviruses, occurred upon colony formation and following parturition. They suggested that the initial peak, upon colony formation, was due to the massing of enough susceptible bats to establish a viral transmission cycle and that the second amplification peak was associated with the introduction of a susceptible subpopulation of newborn pups losing their perinatal immunity. Interestingly, in our Australian study, we also observed two apparent peaks of infection during a three-month study of a maternal colony. Whilst bats occupied this colony irregularly throughout the year, it was upon the formation of the maternity colony that the first peak was observed, coinciding with the observations of Drexler *et al.*[15]. The second peak followed two months later but cannot be conclusively attributed to maternal antibody loss in the subpopulation of newborn pups as none were sampled. It is probable that our inability to sample newborn pups (in an endeavour to reduce stress on them and their mothers) reduced our ability to identify this risk factors responsible for the second peak (loss of perinatal immunity in newborn pups). Also, whilst our study had a very successful recapture rate (82%), the overall sample size (52) was too small and likely precluded us from identifying significant associations for the detection of coronaviruses.

Our identification that individual Australian *Myotis macropus* were infected with a novel putative *Alphacoronavirus* over periods of up to 11 weeks, supports the hypothesis of persistent infection of coronaviruses in some individual bats. Patterns of infection in other individuals are suggestive of intermittent viral shedding (of persistently infected bats) but could also be interpreted as an acute infection (lack of antibody detection in this species precluded distinguishing between the two). While taking care to avoid over-interpretation, a persistently infected bat could become a chronic shedder (as it does for other coronaviruses, including feline coronaviruses). This chronic shedder could potentially be the source of infection to a maternal colony upon its formation.[12,13,20–22].

## Conclusion

When discussing the infection dynamics of bat coronaviruses it would be remiss to ignore the unique biology of bats, the only mammals with the ability for true sustained flight. Flight has previously been linked with viral infection dynamics of bats. It has been suggested that elevated metabolism and body temperature generated during daily cycles of flight was analogous to a febrile response in other mammals and on an evolutionary scale produced a diversity of viruses more tolerant of the fever response[32]. Also, that reactive oxygen species (a by-product of metabolism) placed positive selective pressure on a high proportion of the genes in the DNA damage checkpoint. These flight induced adaptions may have had inadvertent effects on bat immune function and life expectancy[33].

By themselves these adaptations in response to the evolution of flight could have an effect on viral infection dynamics, but the product of flight itself, (general frequent and long distance movement[34,35]) would not only have the effect of spreading the virus into new populations, but would surely have some selective pressure on viruses hosted by bats. Whilst it is reasonable to assume that increased viral prevalence is the result of the congregation of susceptible bats (in a maternal colony or otherwise), conversely, a survival strategy is required for the coronaviruses during periods of its host's dispersal (when flight has afforded the bats the ability to spread out over large areas). Could it also be that whilst bats have adapted to the evolution of flight by controlling the damage of DNA and effects of viral infection, viruses have co-evolved to survive periods of time when susceptible hosts are sparse? Is this the difference that fundamentally drives different transmission dynamics of coronaviruses in bat populations and requires a persistent infection for bat coronaviruses to endure? Jeong *et al.*[36] recently attempted to answer this question by developing epidemic models using the *Myotis macropus* mark-recapture data[17,19], and found that both persistently and transiently infected bats were required for maintenance of coronaviruses.

Together, these studies support the hypothesis for the existence of persistently infected bats and demonstrate an important role that these individuals play in the maintenance of coronaviruses.

A better understanding of a viral transmission cycle is an important step towards breaking it and armed with this knowledge we may be better prepared to prevent the next global pandemic of a bat coronavirus.

## Acknowledgements

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## Biography

After assisting in the surveillance of bats for Hendra virus in Australia, Nipah virus in Malaysia, India and Bangladesh, and for SARS-like coronaviruses in China, **Craig Smith** completed his post-graduate studies at the University of Queensland (Australian bat coronaviruses). He is now a scientist with Biosecurity Queensland, Department of Agriculture and Fisheries, where he recently managed projects for the National Hendra Virus Research Program.