Due to the evolving global medical crisis caused by the COVID-19 pandemic and its current escalation in Australia, the Australian Society for Microbiology Executive has decided to postpone the Annual Scientific Meeting that was due to take place in July 2020.

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Cover image: An image of SARS-CoV-2, the virus responsible for COVID-19 disease. This image is a colourised transmission electron micrograph (TEM) and is work of the Australian Animal Health Laboratory (AAHL), CSIRO.
As we progress through 2020, Microbiology is dominating the news with the emergence and rapid dissemination of the novel coronavirus COVID-19. The impact of COVID-19 on public health, with significant financial, logistical and social repercussions, has quickly become apparent, and is evolving rapidly in Australia. As microbiologists we have an important role to play during this time because we can use our knowledge, expertise and experience to educate the community around us, and to reduce the panic that results from fear and misinformation. It is also critical that we ensure that individuals are not stigmatised because of their perceived role in the transmission of this infectious disease. A coordinated global effort is required to tackle this new infectious threat, and we are an important local part of this effort. Indeed, our public health, medical, teaching and research communities have responded in a remarkable way to protect us against this pandemic, and we are grateful for everything that they are doing.

Unfortunately, due to this evolving global pandemic crisis and its escalation in Australia, the ASM Executive has decided to postpone the Annual Scientific Meeting, CliniCon and EduCon, that were due to take place in July 2020. We did not make this decision lightly and it was essential for us to adopt a responsible stand and to show a duty of care to our members. Executive knows that enthusiasm for the conference had gained momentum as the Local Organising Committee worked hard to build an engaging and exciting program. This was a very hard decision to make. We regret and apologise for this postponement but we look forward to seeing you at our next Annual Scientific Meeting and will communicate our new plans as soon as possible. I would like to thank the local organising committees for their work towards delivering the three events, and to reassure them that their efforts will go a long way towards our future planning.

On a related note, bringing our discipline to the attention of the public and the government at this time is more important than ever. To this end, the chair of our South Australian/Northern Territory Branch, Peter Traynor, has been instrumental in lobbying for the establishment of a Parliamentary Friends of Microbiology group. The Australian Society for Microbiology warmly welcome the reaffirmation of the Parliamentary Friends of Microbiology, in the 46th Parliament, and we gratefully acknowledge the interest of our Federal parliamentarians and their staff in matters pertaining to our discipline, across its broad range of areas. It is intended that this Group will provide a non-partisan forum for MPs to meet and interact with academic, clinical and scientific microbiologists on matters relating to infectious diseases, biosecurity, public health, veterinary and agricultural microbiology, food safety, epidemiology, and research and innovation. We look forward to enabling their knowledge, context and understanding of all matters microbiological, particularly at this important time when infectious disease is dominating news headlines and creating overwhelming community and public health concern. Please see the following link for more information: https://www.aph.gov.au/About_Parliament/Parliamentary_Friendship.

As always, please visit our website www.theasm.org.au to access information regarding upcoming meetings and awards. Note our fresh new website, which is easier to navigate and currently showcases content created by our wonderful ASM Communication Ambassadors. You may also like to follow, and contribute to ASM on Twitter; @AUSSOCMIC, or on Facebook to make sure you keep up with the latest news, trends and developments in Microbiology in Australia and around the world.

**Have you heard of APPRISE?**

It is the Australian Partnership for Preparedness Research on Infectious disease Emergencies. APPRISE says ‘Pandemics are unavoidable’ and lists seven ways their research can save lives.

These include supplying the latest information to decision-makers, investigating the first few hundred cases of each new pandemic, working with communities, improving national and international data sharing, boosting our infectious disease research workforce, improving infection prevention in hospitals and fast tracking trials of new treatments.

APPRISE works on a range of high-impact pathogens, for example, SARS-CoV, MERS CoV, EBOV, and Zika virus.

Check out their website: www.apprise.org.au to see their collaborating institutions, projects, and latest news.
Zoonoses

John S Mackenzie and David Williams

The selection of papers included in this issue of *Microbiology Australia* present a broad brush of zoonotic diseases, from those known or described in ancient times such as rabies, first described in the Eshnunna cuneiform law tablets from ancient Mesopotamia dating back to the 18th–19th centuries BC, and glanders, thought to be first described in donkeys by Aristotle in Ancient Greece in 420–450 BC and subsequently by the Romans, to some discovered or recognised as zoonotic within the past 30 years, such as the recently described zoonotic bat-borne pathogens in Australia, and *Clostridium difficile*, only recently recognised as a zoonotic pathogen. The selection of papers also demonstrates the wide range of zoonotic origins, including arthropod-borne viruses and potentially seafood-borne parasites.

More than 60% of human infectious diseases are caused by pathogens shared with wild or domestic animals, and over 75% of emerging diseases are zoonotic in origin. Over the past few decades, an increasing number of infectious diseases have jumped the species barrier from animals to humans to cause disease, and in many instances have subsequently spread regionally and/or globally. Most of these have been viruses jumping from wildlife to humans, as exemplified by HIV/AIDS in the 1980s originating from the great apes possibly as early as the 1920s; Sin Nombre virus, recognised as a cause of hantavirus pulmonary syndrome in 1993, originating from the deer mouse (*Peromyscus maniculatus*); Nipah virus in 1998–99 originating from bats via pigs; severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002–03 originating from bats via civets; Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 from dromedary camels, but probably originating from bats more than 30 years previously; and currently, the novel coronavirus, SARS-CoV-2, almost certainly originating from bats and probably via an as yet unknown intermediary host, such as pangolins. Other important emerging diseases have jumped from domesticated species, such as pandemic influenza H1N1 originating from pigs in 2009.

What precipitates the cross-species jump, and what can be done to prevent or mitigate it? Much of the recent increase in the emergence and spread of zoonoses can be linked to environmental and societal changes that have brought people and wild and/or domesticated animals closer together, increasing the potential for cross-species transmission. Environmental factors and climate change are altering the habitats of animals or arthropod vectors of zoonoses, changing how and where they live. Anthroponotic factors have changed the interactions between humans and their domestic animals through intensive agriculture and altered land use; and with the need to seek meat for human consumption, there has been increased hunting of wild animals for bushmeat. As cities have expanded and new population centres emerged, there has been increasing encroachment into wildlife habitats. In addition, increased city living and expanding metropolitan areas are providing new homes for a variety of wildlife, from rats and mice to foxes, birds, fruit bats, wallabies, bandicoots, possums, and other small marsupials in Australia, which can live off the plentiful food supply we discard or which are available in parks and gardens, and in additional green areas. In other countries, many local wildlife species are commonly making their homes in cities, including monkeys, squirrels, mongooses and raccoons. Under-scoring this is that ‘synanthropic’ mammal species, those wildlife species that adapt well in human-modified environments, are 15 times more likely to be the source of emerging infectious diseases. As pathogens evolve and emerge, transmission of zoonotic infections and outbreaks have occurred because of the everyday practices of people. This often involves the chain of activities in livestock production, such as intensive growing, breeding, transport, slaughter and sale of animals. In many countries, live (‘wet’) animal markets where several species of domestic or wild animals may be caged in close proximity have been the origins of zoonoses. The interactions of people with wildlife areas for recreational purposes such as hunting, hiking and camping, also lead to zoonotic transmission of pathogens.

Lessons should have been learnt from some of the emergent zoonoses over the past few decades, but despite major outbreaks...
of disease, the memories and messages seem to fail to resonate. The outbreak of SARS in 2002–03 was clearly associated with transmission from live wild animals in the wet markets of Guangdong. Despite strong recommendations that these markets be stopped because of the risks they pose of potential human transmission, they continue to flourish selling poultry and wild and often exotic animals, often illegally (a temporary ban on the trade in wild animals was introduced from 26 January 2020 by the Chinese Government). The same problem exists in the trade and export of bushmeat in Africa. While it is unlikely that the bushmeat trade will be halted in Africa as it provides a much-needed source of dietary protein, export of bushmeat to Europe, US and elsewhere provides an ongoing risk of disease including Ebola, Marburg and other exotic diseases. The amount of bushmeat exported is in surprisingly large amounts, measured in tonnes/airport/year rather than kilograms.

Another recommendation that has not been sufficiently heeded is the need to incorporate surveillance of wildlife disease outbreaks into national and global disease surveillance programs. In Australia, a surveillance system has been implemented to detect outbreaks in free-living wildlife, and the information fed into coordination mechanisms that exist between animal health and public health, and similar systems are in place in the United States and the UK. Wildlife disease surveillance is not well resourced or well reported in many countries, and a concerted and cooperative push is urgently needed to develop improved wild animal disease surveillance mechanisms, especially in resource-poor countries.

Understanding the drivers of human behaviours that lead to the emergence or re-emergence of zoonoses, not just the behaviours themselves, will be equally important to enable comprehensive disease control and mitigation strategies to be put in place. Fundamental to this will be the commitment and support of relevant government departments and industry groups within an affected country to resource zoonotic disease control and prevention through the combined efforts of the human health, livestock and wildlife sectors. Continued efforts to identify potential zoonoses through initiatives such as the USAID Expanded Pandemic Threats program will also be another key element to zoonotic disease preparedness. Closer to home, in Australia, zoonotic disease risks are well recognised, and it will be important for those involved in zoonotic disease diagnosis, research, surveillance and response to remain vigilant and vocal about the ongoing threat they pose. Continued and expanded support for the disease control capabilities of our neighbours in the Pacific and Southeast Asia will also pay dividends for pre-border disease mitigation with the dual benefit of addressing zoonotic disease threats in-country and potentially reducing the importation or spread of pathogens into Australia via people or animals/animal products.

It has been estimated that zoonoses cause about a billion cases of illness in people and millions of deaths every year, and emerging zoonoses are a rising threat to global health, having caused hundreds of billions of US dollars of economic damage over the past 30 years. With the current emergence of the novel coronavirus in China now threatening to develop into a global pandemic, this should surely raise enough concern for a concerted effort to reduce potential opportunities for future zoonosis emergence, using One Health approaches.

References

Biographies

Professor John Mackenzie is an Emeritus Professor of Curtin University, and Honorary Professor in the School of Chemistry and Molecular Biosciences at The University of Queensland. He is a past President of ASM (1992–94), and was awarded Life Membership of the Society in 2019. His recent work has been concerned with global aspects of infectious disease surveillance and response, particularly with respect to emerging zoonotic and vector-borne diseases. He has worked on a number of committees in the World Health Organization, including the Global Outbreak Alert and Response Network, the Asia Pacific Strategy for Emerging Diseases, and was Chair of the first IHR Emergency Committee on Pandemic influenza 2009. He currently serves on the Emergency Committees on the Spread of Poliovirus, and on the Novel Coronavirus Disease, COVID-19. He currently serves on the National Arbovirus and Malaria Advisory Committee. He is also a co-founder of a new foundation to support the concept of One Health, the One Health Platform, based in Belgium. He is also working for one session a week at PathWest in Perth.

Dr David Williams is the leader of the Emergency Disease Laboratory Diagnosis group at the CSIRO Australian Animal Health Laboratory, Geelong, Victoria. This group comprises multidisciplinary capability in virus diagnostics, contributing to national and regional emergency animal and zoonotic disease diagnostics and surveillance. Dr Williams’ research interests have included the detection, diagnosis, and epidemiology of emerging and exotic viruses that affect humans and animals in Australia and overseas. This work has focused on arthropod-borne viruses and has more recently extended to the laboratory diagnosis and pathogenesis of livestock diseases such as African swine fever, Bluetongue and influenza. He is a member of the National Arbovirus and Malaria Advisory Committee and has worked in advisory roles for the United Nations Food and Agriculture Organization and the World Animal Health Organisation (OIE).
The dynamic landscape of bat borne zoonotic viruses in Australia

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This review discusses the history, epidemiology, diagnostics, clinical presentation in humans, as well as control and prevention measures, of the high-profile viruses Hendra virus (HeV) and Australian bat lyssavirus (ABLV). Since the discovery of HeV and ABLV in the 1990s, these viruses have only caused disease in areas where spill-over hosts, including humans, encounter the reservoir host.

Bats

Australia is home to over 90 species of bats, covering many different habitats. All but eight species belong to the suborder Microchiroptera (microbats). See Table 1 for a list of the eight species from the suborder Megachiroptera (megabats) found on mainland Australia, four of which belong to the genus *Pteropus* (commonly called flying foxes or fruit bats). Figure 1 provides a link to an interactive map showing flying fox camps in Australia.

The distribution of bats in Australia has changed over time. As their habitats are destroyed, many have been forced to adapt to life on the urban fringe. There are many successful flying fox camps in the heart of large and smaller cities across Australia – Brisbane, Sydney, Melbourne, Geelong and Cairns to name a few. In the past 10 years, we have seen the southern limit of the black flying fox (*Pteropus alecto*) distribution extend further south, and the south-western limit of the grey headed flying fox (*Pteropus poliocephalus*) distribution extend across into South Australia as well. By contrast, the very small footprint of the spectacled flying fox (*Pteropus conspicillatus*) in far north Queensland, is predicted to get even smaller over time. The black flying fox will most likely fill this void. The ecological drivers behind these changes are complex but are highly likely to include loss of natural habitat, changes to food availability and warming climates.

Hendra virus

Since it was first described in Australia in 1994, HeV has caused horse and human illness and deaths. A high prevalence of neutralizing antibodies to HeV in bats of the genus *Pteropus*, and the isolation of Hendra virus from the same genus, confirmed flying foxes as reservoir hosts for this virus. All four species of pteropus bats can be infected (Table 1). From recent work it appears that the risk of a spill-over event is greatest when either the black flying fox or the spectacled flying-fox is present. The reservoir host appears to co-exist with this virus in complete harmony. The virus spreads easily amongst flying-foxes with the HeV seroprevalence in flying-fox colonies fluctuating over time and geography. The theory of viral co-evolution with chiropteran hosts has been previously suggested, and all field observations and experimental evidence to date supports this hypothesis. Figure 1 provides a link to the results of Hendra virus research conducted in Australia, as well as information for horse owners.

Figure 2 compares the routes of transmission for HeV and ABLV and other closely related bat viruses which result in human infection. For HeV, horses are the main spill-over host and serve as amplifying hosts, capable of infecting humans. The disease in horses exhibits seasonality with more spill-over events occurring in winter. Since it was discovered in 1994, only 95 horses have died to date. Horses in paddocks where flying foxes either roost or come to feed, are at risk of exposure to infection. Infection in horses most likely occurs after close contact with bat urine and birthing material which contain sufficiently high titres of virus to infect a horse.

Extreme care must be taken in the handling of samples collected for HeV diagnostic testing. HeV is a Biosafety level four (BSL4) agent, in...
Table 1. Megachiropteran bats, all belonging to the family Pteropodidae, found on mainland Australia. One common name for each is listed, noting that some have several common names. The last two columns highlight whether evidence of infection with HeV or ABLV has been found in that species.

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>Common name(s)</th>
<th>HeV</th>
<th>ABLV</th>
</tr>
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<tr>
<td>Subfamily</td>
<td>Dobsonia</td>
<td>Dobsonia magna</td>
<td>Bare-backed Fruit Bat</td>
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<td>✓</td>
</tr>
<tr>
<td>Pteropodinae</td>
<td>Pteropus</td>
<td>Pteropus alecto</td>
<td>Black Flying-fox</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pteropus conspicillatus</td>
<td>Spectacled Flying-fox</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pteropus poliocephalus</td>
<td>Grey-headed Flying-fox</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pteropus scapulatus</td>
<td>Little Red Flying-fox</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Subfamily</td>
<td>Macroglossus</td>
<td>Macroglossus minimus</td>
<td>Lesser Long-tongued Fruit Bat</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Macroglossinae</td>
<td></td>
<td>Syconycteris australis</td>
<td>Queensland Blossom Bat</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Subfamily</td>
<td>Nyctimene</td>
<td>Nyctimene robinsoni</td>
<td>Queensland Tube-nosed Bat</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Nyctiminae</td>
<td></td>
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</tr>
</tbody>
</table>

USEFUL RESOURCES

CLICK ICON TO ACCESS WEBSITES WITH INFORMATION

- **ABLV BATS STATS**: A six-monthly report prepared by the WHA Bat Health Focus Group presenting information on ABLV testing in bats.
- **FLYING FOX CAMP CENSUS**: An interactive flying-fox web viewer that presents camp census data collected via the National Flying-fox Monitoring Program.
- **HORSE OWNER INFORMATION**: Advice for horse owners who want to reduce the risk of Hendra virus infection in their horses from the Qld government.
- **NATIONAL HENDRA VIRUS RESEARCH**: Compendium of findings from 20 projects under the National Hendra Virus Research Program, 2016.
- **BAT FAQ**: Answers to questions about flying foxes and possible impacts on human health from NSW Department of Health.

glycoprotein of Hendra virus is very immunogenic and affords protection against HeV challenge in experimental infections\(^\text{17}\). Since the vaccine was released, no vaccinated horse has been diagnosed with Hendra virus infection. Vaccination of horses provides a public health and workplace health and safety benefit by reducing the risk of HeV transmission from horses to humans and other susceptible animals. Whenever HeV infection is suspected, even in vaccinated horses, appropriate biosecurity precautions, including personal protective equipment (PPE), should be used by all people in contact with sick horses.

**ABLV**

In 1996 a five-month-old female black flying fox was found under a fig tree in Wollongbar, NSW, unable to fly. From this bat, a virus with close serologic and genetic relationships to members of the *Lyssavirus* genus of the family *Rhabdoviridae* was isolated\(^\text{16}\). ABLV has since been found in all four flying fox species and in one species of microbat, the yellow-bellied sheath-tailed bat\(^\text{13}\). It is assumed that all Australian bat species have the potential to carry and transmit ABLV. ABLV is transmitted to humans by bites or scratches from an infected bat.

No laboratory tests are currently available to diagnose ABLV in humans before the onset of clinical disease. In the early stages of disease, saliva and cerebrospinal fluid (CSF) can be tested by PCR. Antibody testing can also be performed on CSF. A positive serum antibody test is diagnostic of lyssavirus clinical disease. Any negative test on a symptomatic person is not definitive, as viral shedding in body secretions is intermittent and early tests may be negative for antibody. Therefore, repeat testing is often indicated.

For post-mortem testing in humans and animals including bats, the standard diagnostic techniques include positive fluorescent antibody test (FAT) and PCR on fresh brain smears, and PCR from tissues.

ABLV infection has resulted in three human deaths, two adults and an eight-year-old child, in Queensland, Australia; 1996, 1998 and 2013\(^\text{19}\). Transmission from flying foxes and an insectivorous microbat were implicated, with all three cases displaying features of encephalitic (furious) rabies before their demise. The incubation period is thought to mirror rabies (usually 3–8 weeks, but potentially as short as a few days or as long as several years). Exposure through wounds close to the central nervous system on the head and neck or richly innervated areas like the fingers, carry an increased infection risk and may result in a shorter incubation period. In furious rabies, prodromal symptoms may precede sensorineural dysfunction, with progression to hyperactivity, aperceptua and/or hydrophobia, followed by convulsions\(^\text{20}\). The clinical

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**Figure 2.** Known transmission pathways that result in human infection: the zoonotic transmission pathways of Hendra virus, Nipah virus, Australian bat lyssavirus and rabies virus: (a) Hendra virus: Pteropus bats are the reservoir host. Horses are the main spill-over host, and amplify the virus to very high titres, and succumb to clinical disease and death. From horses the virus can spread to humans if appropriate PPE and other precautions are not taken when handling infected horses and their secretions. Two apparently healthy dogs became infected after exposure to infected horses and experimentally dogs have been shown to be susceptible but unlikely to spread the virus. (b) Nipah virus: Pteropus bats are the reservoir host. Horses can be spill-over hosts and this was seen in one outbreak in the Philippines where humans became infected after eating infected horse meat. The first outbreak of NiV in 1998 had pigs as the main spill-over amplifying hosts and humans involved in pig farming and pig slaughter in Malaysia and Singapore became infected from pigs. In Bangladesh and India there have been almost annual outbreaks and most humans become infected by contact with Nipah virus contaminated date palm sap. Human to human transmission is also seen. While pteropus bats are suspected to be the source of human infection in the most recent outbreaks in Kerala, India, the source of exposure has not been identified. (c) ABLV: Direct contact with infected bats has been the cause of all outbreaks to date, with only humans and horses presenting with clinical signs of infection. Horses have only been infected with a virus from microbats. Two humans have been infected with virus from pteropus bats and one human has been infected from a microbat. (d) Rabies: 99% of all human rabies cases.

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**Human host**

**Reservoir host**

**Amplifying host**

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**Recognition of its status as one of the most dangerous zoonotic agents. Safety precautions during field investigation and in the laboratory are of paramount importance. Blood collected in an EDTA tube, as well as tissue samples from lung, spleen and kidney can be tested by PCR, which is specific for Hendra virus. For the detection of antibodies to HeV in serum either a virus neutralisation test (VNT) or an ELISA can be conducted.

There have been seven known human cases, four of which were fatal. Clinical presentations ranged from self-limiting influenza-like illness, to severe pneumonia and encephalitis\(^\text{16}\). The typical incubation period in humans was 5–21 days, although one person experienced an initial aseptic meningitis, appeared to fully recover, but succumbed to severe encephalitis 13 months later. All human cases had high level exposure to infected horse secretions or tissues\(^\text{16}\). Human to human HeV transmission has not been described to date, unlike the closely related Nipah virus where human to human spread has been reported overseas\(^\text{10}\).

In 2012 a vaccine was released for use in horses, to prevent infection with Hendra virus. This subunit vaccine based on the G protein of Hendra virus is very immunogenic and affords protection against HeV challenge in experimental infections\(^\text{17}\). Since the vaccine was released, no vaccinated horse has been diagnosed with Hendra virus infection. Vaccination of horses provides a public health and workplace health and safety benefit by reducing the risk of HeV transmission from horses to humans and other susceptible animals. Whenever HeV infection is suspected, even in vaccinated horses, appropriate biosecurity precautions, including personal protective equipment (PPE), should be used by all people in contact with sick horses.
course following symptom onset is usually rapid, almost invariably progressing to death within a few days.

Regarding prevention, the key strategy is for untrained and unvaccinated people to avoid handling bats. Public health authorities promote this message particularly during periods of high bat activity, including fruiting periods, and heat stress events when bats and especially pups drop to the ground. Prompt post-exposure vigorous wound cleaning, submission of the bat’s brain for ABLV testing (where possible), rabies vaccination and administration of rabies immunoglobulin, are recommended following bat bites or scratches. Figure 1 provides a link to statistics on ABLV surveillance in Australia, as well as answers to questions about flying foxes and possible impacts on human health from NSW Department of Health.

**Conclusions**

ABLV and HeV can both cause an encephalitis syndrome in humans, sometimes with significant delay or recrudescence. Bats are the reservoirs of these viruses and may well be implicated in transmission of yet to be identified zoonotic pathogens. As the distribution of these reservoir hosts changes, so too does the risk of spill-over events that may involve humans.

**Conflicts of interest**

The authors declare no conflicts of interest.

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**References**


**Biographies**

**Kim Halpin** leads the Pathology and Pathogenesis Group at the Australian Animal Health Laboratory (AAHL). She is a veterinary graduate from the University of Queensland and has worked in research, diagnostic and commercial settings. Her focus has been on emerging infectious diseases. After completing her PhD on Hendra virus, Kim did her postdoc at the Centres for Disease Control and Prevention in Atlanta, USA, working on a Nipah virus reverse genetics project. In 2003 she returned to Australia and conducted henipavirus experimental transmission studies at AAHL. Kim is the OIE Reference Expert for Hendra virus and Nipah virus.

**David Durrheim** is Conjoint Professor of Public Health Medicine, University of Newcastle, and Director - Health Protection, Hunter New England Health. He is a Public Health Physician with an established track record in conducting research that has an operational focus and is translational in nature. Professor Durrheim is an outspoken advocate for equitable global access to effective public health measures, particularly immunisation. He has been instrumental in developing novel surveillance systems to detect and facilitate response to emerging infectious disease risks.
It is unacceptable that as we advance into the 21st century rabies is still a threat to humans and animals alike. Given public health interventions that focus solely on disease prevention in humans have no effect on the reduction of infection in the reservoir hosts, the most effective way to combat human rabies infection is to control the disease transmission by mass vaccination of the animal source, e.g. dogs and wildlife. This short communication focuses on the global strategic target to end human deaths from dog-mediated rabies by 2030 in line with the Sustainable Development Goals by providing recent updates on World Health Organization (WHO) and OIE guidelines and recommendations as well as highlighting Australian rabies research activities to prevent an incursion of rabies into the country.

Dog-mediated rabies is the cause of ninety-nine percent of the 59,000 human rabies deaths annually with the greatest burden being in India and across Africa. Australia has been free of the dog rabies virus variant, although Australian Bat Lyssavirus is maintained in native bats, and has spilled over to horses and people. Travellers to endemic dog-mediated rabies countries should consider the risk of being exposed to the deadly virus and if necessary seek medical advice about pre-exposure prophylaxis rabies vaccination. This risk was sadly evident last year when a 24-year-old Norwegian woman died from rabies having been infected by a puppy she rescued while holidaying in the Philippines. This tragic case highlights the importance of rabies awareness for travellers and communication of preventative measures to reduce the risk of being bitten or scratched by infected animals especially dogs, and appropriate treatment to follow if exposed.

Dog-mediated rabies is an ancient, neurotropic viral disease that should already have been eliminated globally given the tools to control and prevent the disease have been available for decades.

More recently, following the development of the global framework to eliminate dog-mediated human rabies and leadership by the tripartite alliance (WHO, OIE and FAO) and Global Alliance for Rabies Control (GARC), many countries are implementing a multi-sector approach to progress rabies control and elimination. Practical inter-sectoral linking has been successful in the Philippines with 15 island and localities becoming rabies free zones. Additionally, multi-stakeholder national rabies prevention and control committees have supported implementation of national programs.

Only Singapore remains rabies free within the ASEAN region following the reintroduction of dog-mediated rabies to Malaysia during 2015 and 2017 from neighbouring endemic countries. Bali (incursion 2008) continues to progress control of dog-mediated rabies despite challenges in maintaining high dog vaccination coverage across the island. Responsible dog ownership has been highlighted as a key element of National Rabies Elimination programmes in the OIE terrestrial code for rabies although the contribution the control of dog populations plays in dog-mediated rabies elimination remains unknown.

International organisations and updates to rabies guidelines

The WHO Technical Report Series on Rabies No. 1012 released in April 2018, provides new recommendations for pre- and post-exposure prophylaxis, with reduced doses and timing in immunocompetent people. Updates on rabies surveillance are included and cross matched with the revised OIE code on infection with rabies virus. Integrated bite case management is promoted with communication processes developed between human and animal health sectors leading to rapid responses and tracing of infected animals and exposed people. Strengthening of human and animal health systems is necessary to deliver activities of national rabies elimination programs, although often these become less prioritised given competing health emergencies. The WHO and OIE are supporting countries to assess and strengthen their One Health
capacity to deliver zoonotic control programs through Joint External Evaluations (JEE) and IHR–PVS (International Health Regulations–Performance of Veterinary Services) National Bridging Workshops.

The newly introduced progression of countries from endemic rabies to elimination of dog-mediated rabies by implementation of sustained mass dog vaccination programmes and validation and verification of the absence of human deaths from rabies for 24 months was included to support countries reaching zero human deaths. Mexico recently obtained WHO recognition for eliminating dog-transmitted human rabies as a public health issue, adding to evidence that virus transmission can be stopped through mass dog vaccination campaigns.

The OIE Terrestrial Code and Manual for Rabies have recently been through cycles of revisions with the primary tests for rabies diagnosis being direct fluorescent antibody test, direct rapid immunohistochemistry test (dRIT) or lyssavirus polymerase chain reaction assays (PCR) from appropriate brain samples of suspect rabid animals. This testing currently occurs at the Australian Animal Health Laboratories (AAHL) in Geelong for any suspect cases in Australia. Rapid tests (lateral flow devices) are being used in the field to assist in diagnosis although these have variable sensitivity and specificity. Additionally, oral vaccination of dogs is now considered a useful supplementary measure to increase vaccination coverage in the dog population where necessary. The requirements for inactivated and oral rabies vaccines have been revised and updated to be in line with WHO, EMA and FDA provisions. The code chapter on rabies now distinguishes between a country and zone free from infection with rabies virus and from dog-mediated rabies. There is also a new Article on OIE-endorsed official control program for dog-mediated rabies (Article 8.14.11) and for surveillance (Article 8.14.12).

Given no clinical signs or gross post mortem lesions are pathognomonic for rabies, laboratory diagnosis is necessary for suspect case confirmation. To assist neighbouring countries to improve the diagnosis of animal rabies the Australian government supported the development of an immunoperoxidase antigen detection test that could be used in provincial laboratories without the need for expensive fluorescent microscopes. The AAHL has also been building capacity regionally in phylogenetic analysis of rabies viruses to better understand the molecular epidemiology of rabies outbreaks which is especially important during the final stages of dog-mediated rabies elimination. Figure 1 illustrates the different wildlife associated lyssaviruses that may also be circulating in some countries and that can spill over into humans.

**Australian rabies research building surveillance capacity**

With dog rabies spreading into eastern Indonesian islands, and only 300 km from northern Australia shores, pre-border biosecurity and

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**Figure 1.** Phylogenetic analysis of lyssavirus isolates with animals found naturally infected circled. Roman numerals refer to antigenic phylogroups.
active surveillance programs are essential to prevent or rapidly identify an incursion. Risk assessments have focused on estimating the probability of a rabies-infected dog on fishing or recreational boats entering illegally into northern Australian remote indigenous communities and Papua New Guinea. Large numbers of owned dogs are free-roaming in these remote communities therefore research has investigated the roaming behaviour of these domestic dogs (using GPS tracking collars) to better understand interactions to enable modelling of a rabies incursion and interventions. More recent modelling assessed targeted rabies vaccination strategies in different dog populations associated with roaming behaviour. Currently, the optimal rabies population vaccination coverage is seventy percent to achieve herd immunity and prevent virus transmission. The modelling of targeted rabies vaccination strategies based on the roaming behaviour of the dogs (directly associated with risk of rabies transmission) has indicated that lower vaccination coverage may be feasible which is beneficial when rabies vaccines are in limited supply as well as being more cost-effective.

**AUSVETPLAN rabies and Australian bat lyssavirus**

Australia’s national rabies and Australian bat lyssavirus (ABLV) preparedness and emergency response plan (AUSVETPLAN) are currently under review with a joint technical workshop recommending the updated manuals be combined. This is because an emergency response following an outbreak would be strategically similar and require a coordinated response between the public human and animal health agencies. Given the risk of a rabies incursion in northern Australian indigenous communities and the cultural and social importance of dogs and dingo hybrids in these communities, it is recommended that community appropriate strategies for biosecurity responses to an incursion be developed and incorporated into these manuals.

Reporting of potential rabies cases in these remote indigenous communities requires awareness of the disease and participation in this surveillance, which in-turn requires communities to perceive a need for this surveillance. Qualitative studies have explored sustainable community-based surveillance for rabies in these communities and noted the importance of traditional communication channels and direct conversation with valued animal-management services. To communicate rabies risk pathways awareness the Northern Australia Quarantine Strategy (NAQS) have produced an animated video (white paper funded project) for use in community health clinics and schools in northern Australia. Figure 2 shows a diagram from the video illustrating the possible entry points of infected rabid dogs on illegal fishing or recreational boats. The video also promotes awareness about telling a ranger or biosecurity officer about any dogs from boats that are behaving strangely (hypersalivation, paralysis, lethargy, abnormal aggression, abnormal vocalisation). Data on the incidence of dog bites is also important to monitor.

**Conclusion**

The correlating updates on rabies guidelines by WHO and OIE will greatly support the zero by 30 goal and prevention of rabies incursion. Australia’s pre-border biosecurity has successfully facilitated continued freedom from dog-mediated rabies. Ongoing research has built capacity in rabies surveillance and risk assessment and in additional has supported our neighbours in dog-mediated rabies control and, hopefully, eventual elimination.

**Conflicts of interest**

The author declares no conflicts of interest.

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**References**

Sydney’s waterfront, wharves, and buildings were filthy and rat infested. Large areas were quarantined, and rats were caught and exterminated. In fact, the government had distributed tubes, spirit lamp, and glass slides so that cultures and smears of pus and blood could be made at the bedside.

Medical officers called to visit suspected patients equipped with a kit containing syringes, platinum wire loops, culture tubes, spirit lamp and glass slides so that cultures and smears of pus and blood could be made at the bedside.

Point of care testing in Australia began during the first outbreaks of plague in Sydney (1900 and 1902) and Brisbane (1902).

Biography
Dr Andrea Britton is an experienced One Health professional with a strong background in epidemiology, public health and emergency disease preparedness and response. She has global experience with dog-mediated rabies control and eradication as Non-Executive Director for Vets Beyond Borders an Australian non-government organisation and as program officer for OIE (World Organisation for Animal Health) in the southern African region. As a veterinarian with a Master of Public Health degree majoring in Epidemiology, she has a keen interest in developing programs for the prevention and control of zoonotic diseases using a One Health approach. Andrea participated in the revision of WHO Expert Consultation in Rabies (third edition) and the development of the global dog-mediated human rabies elimination framework for ZERO Human deaths by 2030. She has presented internationally in India, Argentina, Canada and South Africa on rabies control and elimination.

Did you know?

Point of care testing in Australia began during the first outbreaks of plague in Sydney (1900 and 1902) and Brisbane (1902).

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Sydney’s waterfront, wharves, and buildings were filthy and rat infested. Large areas were quarantined, and rats were caught and exterminated. Infected persons and healthy people living in the same house were transferred to the Quarantine Station at North Head, now known as Q station and well worth a visit if you are visiting Sydney.

In Focus
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 multidisciplinary ‘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. 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, ultimately leading to the description and characterisation of a bacterium, *Chlamydia psittaci*, with a complex biphasic developmental cycle. 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. 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.

**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 lineages of chlamydiae. 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, a plethora of new species (14 total species in the genus) have now been proposed or formally classified 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.
hosts of chlamydial infections as well as new hosts such as snakes9–12. 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 DNA13. Coupled with the use of the aforementioned broad-range order and family-specific primers8 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 slaughterhouses14–16, 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 workers15. 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 species-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 sera19. 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 species-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 exist19.

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 sequenced20. 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’ strains21.

‘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 cases22. 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 methods23, 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 Wales24. 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 clade25, responsible for the global epidemics in the 1930s and found in Australian native parrots26. 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](image_url)

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.
FUTURE DIRECTIONS IN UNDERSTANDING CHLAMYDIAL ZOONOSIS

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.

Acknowledgements

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References


### Biographies

**Dr Adam Polkinghorne** is a Senior Hospital Scientist at NSW Health Pathology and a Honorary Senior Principle Research Fellow in the University of Sydney Nepean Clinical School. His research interests are primarily focussed on the (a) diagnosis, management and control of chlamydial infections in humans and animals and (b) the detection and control of hospital-acquired infections in infants and at-risk patients.

**Dr James Branley** is an infectious disease physician and Head of Department, Infectious Diseases and Microbiology at Nepean Hospital, Penrith. He is also the Local Pathology Director, NSW Health Pathology Nepean and an adjunct Associate Professor in the University of Sydney Nepean Clinical School. He has a long-standing interest in psittacosis, recently completing a PhD on this topic at the University of Sydney.

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Leptospirosis is a human and veterinary illness caused by spirochete bacteria in the genus *Leptospira*. In symptomatic infection the clinical presentation ranges from non-specific febrile illness to fulminant organ system failure with a high case fatality rate. Leptospires are excreted in the urine of infected mammals, principally rodents, but also dogs, pigs, horses and bats. Many species of wildlife can become infected, including Australia’s iconic kangaroos and Tasmanian devils, and in developing countries in Africa and the Pacific Islands, subsistence livestock are also an important source of exposure. The bacteria can survive for weeks to months in urine-contaminated water and moist soil. Pathogenic leptospires can penetrate mucous membranes, the conjunctiva, or enter through the skin if there are cuts or abrasions. Humans acquire infection either directly via exposure to urine from infected animals, indirectly though contact with urine-contaminated water and wet soil, or by ingestion of contaminated food or water. Human-to-human transmission is very rare but has been documented through sexual contact and breastfeeding.

Limited data from prospective surveillance studies suggest that many human leptospirosis infections are asymptomatic or mild, but the clinical course is highly variable. Symptomatic infection in humans typically appears 5–14 days after exposure, with a range of 2–30 days. The clinical presentation ranges from non-specific febrile illness to fulminant, life-threatening organ system failure.

High-risk activities include both recreational and occupational exposure to surface water and mud that may be contaminated with animal urine. Direct contact with infected animals through farming and slaughtering is also a risk. While leptospirosis occurs worldwide, it is more common in tropical and sub-tropical climates.

Here are some key things to know about the increasing risk to human and veterinary public health posed by this pervasive, but often under-appreciated, pathogen.

(1) **Leptospirosis is a leading cause of zoonotic morbidity and mortality worldwide**

According to the US Centers for Disease Control and Prevention leptospirosis is considered to be the most widespread zoonotic disease in the world. This assertion is supported by modelled estimates from 2015, which found that worldwide there are more than a million symptomatic leptospirosis infections each year.
resulting in almost 60,000 deaths\(^5\). These figures make leptospirosis a leading zoonotic cause of human morbidity and mortality with the number of deaths attributable to leptospirosis approaching or exceeding those caused by viral haemorrhagic fever and canine rabies\(^6\). Given that these estimates were largely derived from hospital-based surveillance studies using passive case ascertainment, they likely underestimate the true global burden of leptospirosis.

(2) The case-fatality rate for severe human leptospirosis illness is high

Most ill persons will experience fever, chills, muscle aches, headaches. Other symptoms may include photophobia, conjunctivitis, anorexia, vomiting, diarrhea, abdominal pain, jaundice, cough, lethargy, arthralgia, calf tenderness, and less commonly, a rash\(^2,3\). Approximately 10 percent of people with leptospirosis progress to severe disease involving one or more of the following: kidney or liver failure, coagulopathy, pulmonary haemorrhage, myocarditis, arrhythmia, shock, optic neuritis, transverse myelitis, meningitis, and encephalitis. Since a broad array of organ systems can be affected, the signs and symptoms are diverse and protean. As a result, leptospiral infections are often incorrectly attributed to other causes of acute febrile illness, such as malaria, dengue, or enteric fever\(^4\). There is now growing recognition that leptospirosis is an important cause of an acute febrile illness in tropical environments, and may be responsible for more than 60% of acute undifferentiated, non-malarial febrile illnesses\(^5\).

Unfortunately, in the case of leptospirosis, misdiagnosis can lead to sub-optimal or delayed clinical management and this contributes to poor outcomes. Early initiation of appropriate antibiotics is associated with a significantly shortened duration of illness and, combined with rigorous ICU-level supportive care (e.g. dialysis, ventilation), can improve the prognosis\(^7\). Still, the case fatality rate for patients with severe clinical illness is very high, ranging from 5 to 15%, a figure comparable to that of meningococcal meningitis.

(3) Challenges in diagnosing leptospirosis directly contribute to its status as a neglected disease

In addition to the difficulty of making a clinical diagnosis, challenges in obtaining laboratory confirmation for leptospirosis contribute to it being under-appreciated as an important human pathogen. Historically, case confirmation has relied on demonstrating antibody seroconversion between acute and convalescent-phase samples using the microscopic agglutination test (MAT)\(^8\). Because the MAT is time-consuming and difficult to perform, various screening tests for detecting IgM antibodies have been developed, but poor sensitivity early in the course of illness means they have limited utility for diagnosing leptospirosis at the time when important therapeutic decisions need to be made\(^8\).

More recently, PCR tests designed to detect the presence of Leptospira DNA in blood (and other tissues) shortly after illness onset have been used in both clinical and research settings for humans and other mammals\(^9-13\). After a 20-year hiatus, leptospirosis was reinstated as a nationally notifiable disease in the United States in 2014 and criteria for a confirmed case includes ‘detection of pathogenic Leptospira DNA by PCR from a clinical specimen’\(^14\). In contrast, human leptospirosis illness has been notifiable in Australia for decades, however, the case definition has not been updated since 2004 and completely omits mention of diagnostic PCR testing\(^15\). Given its utility for diagnosing leptospirosis in the early phases of illness, the case definition used for national surveillance in Australia should be revised\(^15\).

(4) Although it is typically thought of as a ‘tropical disease’, leptospirosis can cause outbreaks in unexpected places

A nearly ubiquitous pathogen, leptospirosis can emerge in some surprising places. The largest outbreak ever reported in the US occurred in 1998 when 110 individuals, most of them athletes competing in a triathlon, were infected in Springfield, Illinois\(^16,17\). Another atypical cluster occurred among inner-city residents of Baltimore, Maryland who were exposed through percutaneous injuries subsequently contaminated by rat urine in alleys\(^18\).

(5) Leptospirosis has caused recent outbreaks among humans and their pets in Australia

Over the 29-year period for which the data are publically available, there have been an average of 149 human leptospirosis illnesses diagnosed and reported in Australia each year (range: 72–319; Figure 1). While the majority (57%) of cases during this period were reported from Queensland, every jurisdiction has had cases\(^19\).

Some of the leptospirosis infections reported in Australia would certainly have been acquired while travelling abroad in high-incidence countries. For persons living in developed countries, activities involving water exposure and eco-tourism are well documented risk factors for acquiring leptospirosis overseas. This underscores the need for clinicians to obtain travel histories on patients presenting with febrile illness and to include leptospirosis in the differential diagnosis for those at risk.

Leptospirosis can also be locally acquired in Australia resulting in sporadic cases and outbreaks\(^20\). In 2018, the largest outbreak of human leptospirosis in Australia occurred in New South Wales when 84 cases were identified over a 5-month period among...
raspberry workers from a berry farm. The farm workers were exposed through scratches obtained while picking raspberries that then became contaminated with leptospires excreted into the environment by infected mice. Three of 13 mice that were trapped as part of the investigation tested positive for Leptospira borgpetersenii serovar Arborea.

In 2019 the first ever outbreak of canine leptospirosis was identified in Sydney, NSW when seven fatal leptospirosis infections occurred among pets in inner city suburbs. Dr Jacqueline Norris, Professor of Veterinary Microbiology and Infectious Diseases at the University of Sydney was quoted as saying, ‘We haven’t seen leptospirosis in Sydney dogs . . . so something has changed’. Displacement of rat populations and pooling water caused by light rail construction works were hypothesised to be potential contributing factors.

Later in 2019, an unprecedented spate of canine deaths was reported from Melbourne, Victoria. According to statements attributed to Victoria’s Chief Veterinary Officer, three fatal canine leptospirosis infections were reported from eastern Melbourne, a phenomena that veterinarians who treated the dogs said they had never seen before. However, serological evidence of canine leptospirosis in Australia suggests that the disease is more widespread than previously appreciated. A serosurvey of almost 1000 dogs sampled at shelters in Queensland, New South Wales, Victoria, Western Australia and the Northern Territory found that 1–2.5% were seropositive, with Leptospira interrogans serovar Copenhageni being most prevalent of the 11 different serovars detected. Whether the recent outbreaks of leptospirosis among dogs in major Australian cities are simply a ‘one-off’ event or signal a growing urban hazard remains to be seen.

(6) Climate change will likely increase the risk of future leptospirosis outbreaks

Extreme weather events resulting in flood-related disasters are predicted to increase with climate change. Flood-related leptospirosis outbreaks have already been documented in many parts of the world, in both developing and developed countries, including Australia, and may be becoming more common. Pacific Island countries already have the highest leptospirosis disease burden in the world and experience frequent outbreaks. Improved surveillance for leptospirosis in human and veterinary medicine will be important for assessing and responding to the impact of climate change on this prevalent, but still emerging, zoonosis.

(7) Australia is well positioned to help address emergence of leptospirosis locally and internationally

Australia has one of the best leptospirosis laboratories in the world. The Leptospirosis Reference Laboratory in Queensland is accredited by the World Health Organization and the Office International des Epizooties (OIE). This laboratory provides expert advice and diagnostic support to public health authorities at local, national and international levels, and collaborates extensively with universities and government departments to support research and surveillance in the human and veterinary fields. Australia is also fortunate to have experienced clinicians and researchers whose expertise in leptospirosis is respected globally; the work of some of these individuals is cited in this article.

In addition, Australia has been a leader in advocating for a One Health approach to address zoonotic infectious diseases. One Health recognises that developing effective strategies to mitigate the threat to human and animal health posed by zoonotic pathogens will require the engagement of professionals with a diverse set of skills and from a range of disciplines. This approach will be critical to fully understanding the emergence of leptospirosis in an era of climate change, population growth, changes to agricultural practices, increased travel and urbanisation. Fortunately, given its expertise and laboratory capacity, Australia is well positioned to

Figure 1. Number of notifications of Leptospirosis, Australia, by jurisdiction and year, 1991–2019.
make significant contributions, both at home and abroad, for responding to the threat posed by this quintessential – but often neglected – zoonotic pathogen.

Conflicts of interest

The author declares no conflicts of interest.

Acknowledgements

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References


Biography

Paul Effler received a Doctorate in Medicine from the University of California and a Master of Public Health from the University of Hawaii. On completing a residency in Public Health Medicine, Dr Effler served as an Officer in the Epidemic Intelligence Service at the US Centers for Disease Control. In 1994 he became the State Epidemiologist for Hawaii, where he directed the public health response to leptospirosis and other communicable diseases. In 2008 Dr Effler moved to Perth where he works in communicable disease control. He is a Clinical Professor at the University of Western Australia School of Medicine, an Associate Editor for Emerging Infectious Diseases, and serves on the Steering Committee for the Global Outbreak and Response Network and the Technical Advisory Group for the Asia Pacific Strategy for Emerging Diseases and Public Health Emergencies with WPRO/WHO.
The One Health concept recognises that the health of humans is interconnected to the health of animals and the environment. It encourages multidisciplinary communication and collaboration with the aim of enhancing surveillance and research and developing integrative policy frameworks. *Clostridium difficile* (also known as *Clostridoides difficile*) infection (CDI) has long been viewed as a hospital-associated (HA) enteric disease mainly linked to the use of broad-spectrum antimicrobials that cause dysbiosis in the gut and loss of ‘colonisation resistance’. However, since the early 2000s, the rate of community-associated CDI (CA-CDI) has increased to ~15% in Europe, ~30% in Australia and ~40% in the USA in populations often without obvious risk factors. Since the 1990s, it has become apparent that food animals are now a major reservoir and amplification host for *C. difficile*, including lineages of clinical importance. Cephalosporin antimicrobials, to which *C. difficile* is intrinsically resistant, were licensed for animal use in North America in 1990. By the second decade of the 21st century, there were reports of *C. difficile* contamination of food and the environment in general. Using whole-genome sequencing (WGS) and high-resolution typing, *C. difficile* isolates from humans, animals, food and the environment were proven to be genetically closely related and, in some cases, indistinguishable. This suggests possible zoonoses and/or anthroponoses, with contaminated food and the environment acting as the conduit for transmission between animals and humans. This paper summarises the key evidence that demonstrates the One Health importance of *C. difficile*.

The role of asymptomatic carriers in the spread of *C. difficile*

In 2013, the landmark study of Eyre *et al.* provided the first compelling evidence that asymptomatic carriers, and possibly other unknown sources external to the healthcare setting, were playing a major role in *C. difficile* transmission. The study, conducted in Oxfordshire in the United Kingdom used WGS and core-genome single nucleotide variant (cgSNV) analysis to examine *C. difficile* strains from 957 hospital- and community-identified CDI cases collected between 2008 and 2011. The authors found only 333 isolates (35%) had a clonal relationship (<2 SNVs difference) with isolates of the same PCR ribotype (RT) from other CDI cases, and 428 isolates (45%) were genetically distinct with >10 SNVs difference in their core-genomes. Of the 333 cases with evidence of clonal transmission, only 126 (38%) had close hospital contact with another CDI patient and 120 (36%) had no plausible epidemiological link to a patient in the hospital system or community.
In early 2019, Sheth et al.\(^2\) and Halstead et al.\(^3\) presented more evidence for asymptomatic carriers playing a part in the dissemination of \textit{C. difficile} in hospitals. In both studies, asymptomatic patients were screened for \textit{C. difficile} on admission and isolates were compared to isolates from symptomatic CDI patients using Multi-Locus Variable Number Tandem Repeat Analysis (MLVA) or cgSNV analysis. Of the 10–15% asymptomatic patients that tested positive for \textit{C. difficile}, >80% were colonised by toxigenic \textit{C. difficile} strains capable of causing disease.\(^2,3\) These studies revealed \textit{C. difficile} transmission from asymptomatic patients to previously \textit{C. difficile}-negative patients,\(^5\) and clustering of asymptomatic patients with symptomatic CDI patients,\(^3\) supporting the idea that asymptomatic carriers are spreading \textit{C. difficile}. Furthermore, in a separate study by Gonzalez-Orta et al.\(^4\), 27% of HA-CDI cases in Cleveland, USA, were infected with strains that the patients were previously colonised with on admission. This suggests that they were not true HA cases and that \textit{C. difficile} was likely acquired in the community, with disease manifesting only after admission to hospital. With continuous importation of \textit{C. difficile} into the hospital setting via asymptomatic carriers, community reservoirs are undoubtedly playing a much bigger role in the transmission of CDI than previously thought and the incidence of CA-CDI might have been grossly underestimated using the current guidelines.\(^5\)

### Community reservoirs

To date, \textit{C. difficile} has been isolated from diverse array of sources/reservoirs including food animals (pigs, cattle, sheep and poultry), meat (veal, beef, pork, lamb, chicken and turkey), seafood (clams, salmon, shrimp and mussels), vegetables (lettuce, pea sprouts, ginger, carrots, potatoes and salad), the household environment (toilets, floors, bathroom sinks and soles of shoes) and the natural environment (rivers, lakes and soil).\(^6\) In summary, food animals, retail food and the environment are important reservoirs of \textit{C. difficile}. The average prevalence of \textit{C. difficile} in neonatal animals is always high, ranging from ~20% in calves to ~70% in piglets.\(^6\) \textit{C. difficile} prevalence as high as 42% in retail meat has been reported in the USA;\(^7\) however, European studies reported a much lower figure of ~3%,\(^8\) possibly related to differences in slaughtering practices. Meanwhile, the prevalence of \textit{C. difficile} in natural environments such as soil and water averages ~30%.\(^6\) The most common strains identified in these studies are \textit{C. difficile} RT 014, belonging to multi-locus sequence types (MLSTs [STs]) 2, 13 and 49, and ST11 RTs 078, 126, 127 and 033. All these strains are toxigenic, associated with human CDI, well established in multiple animal and environmental sources and invariably resistant to numerous antimicrobials used in human and veterinary medicine.\(^9,10\) This further demonstrates the relevance of \textit{C. difficile} to the One Health concept, i.e. there are three independent yet convergent problems that require an integrative solution: a human health issue, an animal health issue and an environmental issue.

### Long-range interspecies transmission of \textit{C. difficile}

To date, there has been no incontrovertible proof of foodborne or environmental transmission of \textit{C. difficile}. Such proof remains elusive given \textit{C. difficile} is not a typical foodborne or enteric pathogen: (1) not all individuals exposed to \textit{C. difficile} will develop symptoms (depending on the vulnerability of their gut microbiota); (2) \textit{C. difficile} is ubiquitous in the environment; and (3) the usual rules for source attribution are often not obeyed.\(^11\) Nevertheless, largely due to the advent of microbial genomics, there is now ample evidence that: (1) \textit{C. difficile} common to humans and production animals share a recent evolutionary history; and (2) CDI has a substantial zoonotic component which results in the spillover of \textit{C. difficile} into retail food and the environment. Building on their earlier work showing clonal transmission of \textit{C. difficile} between a pig and a pig farmer,\(^12\) Knetsch \textit{et al.}\(^13\) sequenced 247 \textit{C. difficile} RT 078 strains from diverse sources in 22 countries across four continents (North America, Europe, Australia and Asia). Core-genome analysis revealed extensive clustering of human and animal strains, evidence of potential bidirectional spread of \textit{C. difficile} between farm animals and humans. There was limited geographical clustering with clones of \textit{C. difficile} RT 078 spread across towns, countries and continents. One clonal group of RT 078 showed intercontinental transmission between an animal in Canada and humans in the United Kingdom. Another study\(^10\), this time focusing on a global population of ST11, corroborated the findings of Knetsch \textit{et al.}\(^15\) revealing a globally disseminated network of \textit{C. difficile} ST11 clones (of RTs 078, 126, 127, 033 and 288) with the propensity for reciprocal zoonotic and/or anthropogenous transmission. Tetracycline use in agriculture and animal husbandry is widespread and its inappropriate use in the latter is well recognised. Dingle \textit{et al.}\(^14\) found tetracycline selection to be a key driver of \textit{C. difficile} RT 078 evolution, with multiple independent tetM-associated clonal expansions of this lineage occurring around the year 2000. Further supporting an agricultural focus for \textit{C. difficile} RT 078, the evolutionary origins of these different tetracycline resistance elements were Tn916-like elements (which are capable of inter-species transfer) from established zoonotic species including \textit{Streptococcus suis}, \textit{Enterococcus faecalis} and \textit{Escherichia coli}.
RT 014 is the most successful *C. difficile* lineage worldwide. In Australia, this RT 014 is well established in humans with CDI and pigs, accounting for around 30% and 25% of isolates, respectively.\(^{13-17}\). Knight et al.\(^{7}\) sequenced a contemporaneous collection of *C. difficile* RT 014 strains of human and porcine origin and cgSNV analysis revealed recent interspecies transmission, with 42% of human isolates having a clonal relationship with at least one animal isolate. Again, these clones were isolated months and thousands of kilometres apart across different States of Australia. Thus, it is unlikely that there was any direct contact between the animals and humans, however, it appears that *C. difficile* frequently moves between food animals and humans and that the zoonotic spread is not confined to any geographical region or local population. This strongly suggests an interconnected long-range zoonotic and/or anthropogenic transmission pathway involving recycled waste-products such as manure, biosolids and compost which could result in contaminated crops and/or widespread dissemination of *C. difficile* in the environment. Indeed, studies have shown that retail meat, vegetables, compost, public lawn, household environment and companion animals are reservoirs of clinically important and often antimicrobial-resistant (AMR) *C. difficile* lineages, including RT 014\(^{7}\). This is also in agreement with a WGS study involving 482 European hospitals which revealed no within-country clustering for RTs 078, 015, 002, 014 and 020, consistent with Europe-wide dissemination\(^{18}\).

**Transmission cycle**

How does *C. difficile* spread between food animals and humans with limited geographical clustering? The principal amplification hosts of *C. difficile* are animals, both human and non-human. *C. difficile* from food animals can contaminate meat during processing at the slaughterhouse and survive up to the point of human consumption as *C. difficile* spores can endure the recommended cooking temperature for meat (71°C) for over 2 h\(^{19}\) as well as freezing, chilling and disinfection processes\(^{20,21}\). *C. difficile* spores can also disseminate in the air, in hospitals\(^{22}\) and animal production facilities\(^{23}\). Transmission by invertebrate vectors also occurs. Depending on local agricultural practices and policies, manure from food animals can either be composted or applied directly onto farmland as fertiliser resulting in contamination of the farming environment. Even if the manure is composted, complete elimination of *C. difficile* spores is unlikely; ~60% of composted products such as garden mixes and mulches are contaminated with *C. difficile* (Lim et al. unpublished). Contaminated food waste can also be composted for use in gardening and landscaping. *C. difficile* can survive the process of sewage treatment\(^{24}\) and release of treated sewage effluent can impact rivers and marine life\(^{25}\). While direct zoonotic transfer of *C. difficile* between pig farmers and pigs has been reported\(^{12}\), for the general public indirect zoonotic transmission through food and the environment is more likely. With *C. difficile* being so widely disseminated in the community, household environments and companion animals are also being contaminated/colonised with *C. difficile*\(^{26}\), providing yet another route for CDI transmission. Figure 1 shows the major reservoirs and known transmission pathways of CDI\(^{27}\).

**Conclusions**

In summary, *C. difficile* is a pathogen with substantial community reservoirs and evidence of long-range interspecies transmission. This appears to be a recent (in the past 50 years) event, likely linked to anthropomorphic factors such as high-intensity animal husbandry, international travel and trade and, most critically, injudicious antimicrobial usage in farm animals. High-resolution One Health-focused surveillance of *C. difficile* from diverse human, animal and environmental sources will continue to be critical to the development of a better understanding of the epidemiological and genetic factors contributing to the emergence, evolution and spread of CDI. The control of CDI is currently focused on antimicrobial stewardship and infection control around CDI patients in the hospital setting. With the new knowledge of asymptomatic carriers spreading *C. difficile* in hospitals, early screening and isolating *C. difficile* carriers on hospital admission could help prevent HA-CDI as suggested in a recent Canadian study\(^{28}\), which saw the incidence of HA-CDI decrease significantly from 6.9 to 3.0 per 10 000 patient-days, a 62.4% reduction in expected CDI cases. However, if we are to make meaningful interventions which impact upon both human and animal health, it is imperative that we move beyond the hospital setting and foster collaborative relationships between industry, government, veterinarians, clinicians and researchers. Enhanced antimicrobial stewardship in both human and veterinary settings is crucial but a more productive approach in reducing CDI would be to minimise the environmental burden of *C. difficile* by reducing carriage/infection in both animals and humans with a vaccine. Despite the recent demise of the Sanofi *C. difficile* vaccine program\(^{29}\), several other contenders remain in the pipeline including Pfizer who are currently conducting a phase III trial of a vaccine based on genetically and chemically detoxified toxins A and B\(^{30}\). In addition, a vaccine that offers protection against both CDI and colonisation (via mucosal antibodies to reduce the adhesion of *C. difficile* to mucus-producing intestinal cells) is currently being tested by GSK in a phase I clinical trial\(^{31}\). *C. difficile* is already considered a critical AMR pathogen by US Centers for Disease Control and Prevention\(^{32}\) and should also be...
recognised as the most significant One Health problem in the world today.

**Conflicts of interest**

The authors declare no conflicts of interest.

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**References**


**Figure 1. Transmission pathways for *C. difficile***

*In Focus* 

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An endemic disease is one that exists permanently in a particular region or population. Not usually seen by the media or the general public as a cause for concern even though it quietly and consistently claims lives.

An outbreak is the sudden occurrence of a disease in an unexpected location and/or in group in numbers greater than expected. Seen as a possible news story and somewhat annoying but usually easily forgotten unless it evolves . . . into an epidemic.

An epidemic disease is one that suddenly involves a greater number of people than usual. Seen as undesirable and unpleasant, prompting better hand hygiene by some. It too can evolve . . . into a pandemic.

A pandemic disease is one that causes epidemics worldwide. Usually greeted with horror, mild hysteria and sometimes even panic. The cause is suddenly a matter of grave concern, a killer, and the situation is very serious. Masks may be seen as mandatory street gear, worn for days without changing.
Rift Valley fever (RVF) is a mosquito-borne viral disease, principally of ruminants, that is endemic to Africa. The causative Phlebovirus, Rift Valley fever virus (RVFV), has a broad host range and, as such, also infects humans to cause primarily a self-limiting febrile illness. A small number of human cases will also develop severe complications, including haemorrhagic fever, encephalitis and visual impairment. In parts of Africa, it is a major disease of domestic ruminants, causing epidemics of abortion and mortality. It infects and can be transmitted by a broad range of mosquitos, with those of the genus *Aedes* and *Culex* thought to be the major vectors. Therefore, the virus has the potential to become established beyond Africa, including in Australia, where competent vector hosts are endemic. Vaccines for humans have not yet been developed to the commercial stage. This review examines the threat of this virus, with particular reference to Australia, and assesses gaps in our knowledge that may benefit from research focus.

**Epidemiology and ecology**

Epizootics of RVF occur at irregular intervals, with inter-epizootic periods often spanning years or decades. The survival and re-emergence of the virus after long periods of quiescence is thought to occur through transovarial transmission, but likely also by low-level transmission between mosquitos and a wildlife reservoir. Continuous low-level transmission also occurs within domestic livestock populations without noticeable disease or abortions. RVF virus has been isolated from numerous mosquito species, but certain *Aedes* species associated with freshly flooded temporary water bodies are regarded as maintenance vectors, while *Culex* species associated with permanent fresh water are regarded as epidemic or amplifying vectors.

Rift Valley fever has been isolated from a wide range of mosquito species but laboratory vector competence studies on African mosquitoes support the epidemiological importance of only a few specific *Aedes* and *Culex* species. Other species have been shown to be susceptible to infection but poor at transmitting the virus. A single study evaluated vector competence of Australian *Aedes* and *Culex* mosquitoes for RVFV, with high rates of infection noted, and the ability...
to transmit the virus efficiently after intrathoracic inoculation or oral exposure.\(^{16}\)

**Genome and taxonomy**

Rift Valley fever virus is the only described member of the type species of the *Phlebovirus* genus, *Rift Valley fever phlebovirus*, classified in the family *Phenuiviridae*, order *Bunyavirales\(^{17}\)*. The virus’s relatively stable RNA genome, a result of alternating infection between arthropod and vertebrate hosts\(^ {16}\), consists of two negative-strand segments and a third segment utilising an ambisense coding strategy. The negative sense large and medium segments encode the polymerase and precursor glycoproteins respectively\(^ {19,20}\), while the small segment encodes the nucleoprotein in the negative sense and a non-structural protein in the positive sense\(^ {21}\). This non-structural protein (NSs) is the major virulence factor of the virus due to its ability to counteract host innate immune responses by acting as an interferon antagonist\(^ {4}\). Development of experimental live attenuated vaccines for RVF exploits this knowledge, following the discovery of a naturally attenuated avirulent isolate, clone 13, that has a large deletion in the NSs coding gene\(^ {22}\).

**Diagnostics**

Laboratory confirmation requires positive results from a combination of at least two different diagnostic test methods, which includes virus detection and serological assays\(^ {23}\). Laboratory confirmatory testing is complicated by biocontainment requirements and potential use as a bioweapon, thereby limiting testing to a small number of reference laboratories in the world. Technically, however, laboratory testing is relatively simple due to the low genetic variability of the virus\(^ {4}\) and the existence of a single known serotype. Virus isolation in suckling mice or cell culture and demonstration of a neutralising antibody response by microneutralisation test or plaque-reduction neutralisation test remain the gold standard methods for virological and serological diagnosis respectively, but both require virus propagation, thus necessitating high biocontainment facilities. Safer alternatives have, however, been adapted by most laboratories and are used as first line assays. Molecular assays such as real-time RT-PCR or loop-amplification mediated PCR (LAMP) are mostly used for detecting acute infections\(^ {24,25}\), although antigen detection ELISAs have application for certain sample types\(^ {26}\). Various ELISA platforms have been developed and shown to be sensitive and specific for detection of antibodies to the virus in various species\(^ {27,28}\), including some based on recombinant viral proteins that do not require biocontainment facilities for production\(^ {29}\). Proper validation of assays using clinically relevant material in sufficient numbers remains a challenge and often depends on laboratory generated positive material. There is also no well established internationally available external quality assurance or proficiency testing scheme for either serological or molecular diagnosis of RVF, particularly in endemic African countries, apart from some *ad hoc* studies that are mostly opportunistic and dependent on funding availability\(^ {30,31}\).

**Pathology and pathogenesis**

Human infections, which are usually acquired from contact with infected animal tissues, and thus are an occupational risk for veterinarians, farm worker and abattoir workers\(^ {32}\), manifest as sub-clinical infection or mild febrile illness\(^ {33,34}\). However, in a small number of cases the infection develops to cause severe disease, which may take the form of a haemorrhagic fever syndrome, encephalitis, retinal degeneration or other complications. The impact of these forms of the disease are usually severe with high mortality or long-term impairment of neurological function and sight. In the initial phase of the disease, 1–4 days after infection, there is a viraemia, which declines as antibody levels rise. Related to the viraemia is a vasculitis, which leads to thrombosis and other vascular complications, and these often manifest days to weeks after the initial infection. Infection of the liver is an important component of infection in highly susceptible species such as sheep and mice; this develops during the acute infection stage and may become the dominant pathological feature.

RVF haemorrhagic fever syndrome is characterised by haemorrhage and multi-organ failure and is caused by fulminant hepatic necrosis and vasculitis, two processes that lead to disseminated intravascular coagulopathy through non-renewal (hepatic necrosis) and depletion (vasculitis) of clotting factors. Clinical signs include vomiting, bleeding from the gums, conjunctivae and other mucous membranes, haematemesis, subcutaneous haemorrhages and jaundice\(^ {34,35}\). Severity of disease has a strong correlation with viral load, cytokine responses and coagulation pathways\(^ {36,37}\). Encephalitis may develop in a small proportion of cases some days or weeks after the initial febrile episode and its clinical presentation may depend on the localisation of infection foci in the brain\(^ {33,34}\). On histopathological examination there is a focal necrosis with mono-nuclear cell perivascular cuffing\(^ {35}\). Encephalitis usually occurs despite the presence of antibodies to RVFV, implying that the condition is due to immunologically mediated damage in response to residual infection. Recovery, like many viral encephalitides, can be long and of variable outcome.

Retinal degeneration is probably a sequel to local ocular vascular thrombosis, appearing during the initial febrile disease or up to...
Sheep, and young lambs in particular, are highly sensitive to RVFV infection. Typically, the first sign of infection in a herd is signalled by abortions, and this can be very high with up to 100% of pregnant ewes losing their lambs. Abortion is the outcome of infection of multiple foetal tissues, including the foetal–maternal interface of the placenta. Infected lambs that survive to term are weak and usually do not survive longer than a few days. Viraemia in experimentally infected animals occurs from day 1–7 after inoculation, with peak viraemia around day 2 after inoculation. While the frequency of severe illness and death in adult sheep is lower due to their relative resistance, adults may nevertheless often develop fatal illness, caused principally by hepatic necrosis, vasculitis and associated disorders. Clinical signs include abortion, lethargy and weakness, congested mucous membranes and bloody diarrhoea. The principal lesions include hepatic necrosis, vasculitis, renal tubular necrosis and lymphoid necrosis.

The disease in other ruminants can be similar, but usually less severe, to that in sheep. Abortion in pregnant cattle, goats and camelds is the most common outcome of infection, while young animals tend to be highly susceptible. Rodents and non-human primates are used as laboratory models to study infection and vaccination in humans.

Control

There are no registered human vaccines for RVF and the commercial prospects for such a vaccine remain unlikely unless the virus were to become more widespread and epidemics more frequent. Animal vaccines mainly consist of inactivated cell culture vaccines. The naturally attenuated clone 13 has been investigated as a potential safe vaccine candidate for livestock use, but a recent study found that although it is safe for use in lambs, the virus is able to cross the placental barrier and cause malformations and stillbirths, thereby excluding its use during the first trimester of gestation in sheep. While they are commonly applied in those areas of Africa where the virus occurs, the long inter-epidemic periods frequently lead to complacency in their use. Therefore, there is a clear need for research into vaccine development. Research into the application of rapid scalable manufacture methods, for both human and animal vaccines, would be valuable to areas of the world that do not currently have the agent. For the African situation, there will also be value in more accurate epidemic forecasting, to assist farmers in planning their vaccination schedules prior to the beginning of outbreaks. Mixed vaccine formulations, adding RVF vaccine into vaccines targeting more common veterinary diseases, may help eliminate poor vaccination coverage due to complacency during inter-epizootic periods.

Concluding remarks

The competence of Australian mosquito vectors to become infected with and transmit RVFV indicates a potential risk if the virus were to be introduced. However, establishment of autochthonous transmission depends on more factors than vector competence, for example vector and susceptible host density and distribution, vector behaviour (zoophilic vs anthropophilic), climatic factors such as rainfall and temperature and agricultural practices. RVFV introduction into Australia would present a major challenge to both veterinary and public health authorities. Although RVF is listed as an arbovirus of importance by both the Departments of Agriculture and Health in Australia, very little if any research relevant to the Australian landscape has been published. Potential areas of importance could include an updated study on the competence of Australian mosquitoes for RVFV transmission, combined with detailed mapping of distribution and density of mosquitoes found to be potentially important in transmission. Such information would contribute to modelling disease transmission and thereby contribute to risk assessment and development of mitigation strategies. A multidisciplinary approach would enrich this data by including other factors shown to be important in the ecology of RVF, such as climate, vegetation and soil. To improve confidence in assay performance, baseline serological surveys in targeted areas of the country could be important, or at the very least provide panels of known negative sera to determine serological assay specificity estimates for specific Australian livestock populations. Susceptibility of common Australian livestock and wildlife species should be determined through experimental infection studies.

Conflicts of interest

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**Biographies**

**John Bingham** is a veterinary pathologist at the CSIRO Australian Animal Health Laboratory, based in Geelong, Victoria. His particular interests include the pathogenesis of viral infections in animals. His
team works on diagnosis and response of emerging infectious diseases and the pathogenesis of viral diseases using advanced cell culture systems and animal models. The pathogens he has studied during his career include many of the Biorisk group 3 and 4 viral pathogens, such as lyssaviruses, Hendra virus, highly pathogenic avian influenza virus and infectious prion diseases.

Petrus Jansen van Vuren is a Research Scientist working on transboundary animal diseases at the CSIRO Australian Animal Health Laboratory since mid-2019. For 13 years prior to joining the CSIRO team, Petrus was a Medical Scientist at the National Institute for Communicable Diseases in Johannesburg, South Africa. During this time through his role as BSL-4 laboratory supervisor for eight years and head scientist of the Arbovirus Reference Laboratory for four years, his research focussed on development of diagnostic assays for viral zoonotic diseases, ecological and epidemiological studies of arthropod borne- and haemorrhagic fever viruses, pathogen discovery and mobile laboratory outbreak response capacity.

Petrus obtained a PhD in Virology in 2011 from the University of the Witwatersrand in South Africa after completing research on diagnostic assay and vaccine development for Rift Valley fever. He has authored more than 50 peer-reviewed publications in international journals, two book chapters on haemorrhagic fever viruses, and contributed to the OIE Terrestrial Manual on Crimean-Congo haemorrhagic fever. Petrus has been actively involved in technical training on VHF and arbovirus laboratory diagnostic techniques at African institutes through programs offered by the World Health Organization, International Atomic Energy Agency and the FAO. Petrus contributed to the establishment and management of the first African institute-led mobile diagnostic laboratory in Freetown, Sierra Leone, during the peak of the Ebola haemorrhagic fever outbreak in West Africa between 2014 and 2016. Petrus holds a G2 researcher rating from the National Research Foundation of South Africa, as an established researcher enjoying international recognition of research contributions.
Seafood is an increasingly popular source of healthy protein. Since 1961, the average annual increase in global food fish consumption has been twice as high as population growth and exceeds the consumption of meat from all terrestrial animals combined. The following overview of seafood safety concerns is intended to help readers to understand potential risks associated with parasites in seafood products and the need for a national approach to reduce or minimise them. It is important to note that parasite infections are not limited to seafood: all other types of foods, including vegetables and red meat can also be infected with a broad range of parasites, some of which are more dangerous than parasites in seafood.

Parasites and seafood

The knowns

Occurrence in seafood: A wide range of parasites transmissible to humans can be found in seafood products. Their life cycle, pathogenicity and significance has been reviewed previously. Of over 40 transmissible parasites from seafood reported worldwide, this article will focus more on nematodes belonging to families Anisakidae, Raphidascarididae and Gnathostomatidae, which seem to be of most concern in Australia due to their common occurrence, infecting seafood products in local and global food supply chains. Recent studies showed that 86% of tiger flathead, 56% of anchovy and 100% of pilchard sold in a fish market in Australian east coast were infected with at least one type of infective stage of potentially zoonotic anisakid/raphidascarid nematodes. These nematodes included Anisakis, Contracaecum, Hysterothylacium and Terranova larval types, all of these genera known to have species infecting humans. All of these parasites are now known to migrate from the internal organs of the fish into other parts, including the flesh, after the fish is caught/dead, hence increasing the risk they may pose to public health. Migration of parasites into fish flesh can be minimised by evisceration of fish immediately after capture and appropriate cold storage at all times preceding consumption. Pre-freezing is recommended if fish are intended to be consumed raw or partially cooked. While a diverse range of potentially zoonotic nematodes have been found in Australian wild caught marine fish, little is known about their diversity and abundance in the freshwater fish species in Australia.

Occurrence in humans: With the frequent occurrence and abundance of these parasites, the popularity of seafood, the multicultural cuisine enjoyed in Australia and the ready availability of a variety of seafood, it is not surprising that human infections can occur regularly. In Australia, since the early 20th century, there have been numerous documented cases and many anecdotal cases of human infection with a broad range of parasites acquired from seafood. This not only includes nematodes, such as Contracaecum sp., Gnathostoma sp. (possibly a misdiagnosed case, see Shamsi and Sheorey) and Dicrocoelium dendriticum, but also other parasites including tapeworms, such as Adenocephalus pacificus, Spargana spp., and Diphyllobothrium latum (now accepted as Diplobothrium latus), flukes such as Clonorchis sinensis and Paragonimus westermani, and Myxosporidians such as Myxobolus electrophilus. The reported parasites include both marine (e.g. Adenocephalus pacificus) and freshwater (e.g. Clonorchis sinensis and Paragonimus westermani) parasites. However, due to a decline in parasitology teaching in the Australian medical schools, many of these species of zoonotic parasites have fallen into obscurity.
Clinical signs: Diseases due to some seafood-borne parasites can be severe to life threatening. However, many are mild, perhaps underdiagnosed, and hence not well documented. Clinical signs due to seafood-borne parasites of concern in Australia (anisakids/rhabdascarids and gnathostomids) are broad mainly due to the wide range of species implicated in infections, each parasite species having unique characteristics. Clinical signs, particularly those caused by anisakid/rhabdascarid nematodes, are varied and include: (1) mild vague symptoms that mimic food poisoning; and (2) acute gastrointestinal symptoms that mimic appendicitis. In addition, (3) infection/exposure with anisakid nematodes, even those killed in well-cooked seafood, can also cause allergic reactions, including anaphylactic shock in sensitive consumers and workers handling seafood products. Gnathostomid nematodes are also migratory parasites which cause highly variable clinical signs. They invade a variety of tissues, including subcutaneous, pulmonary, gastrointestinal, genitourinary, auricular or ocular tissues, or the central nervous system with migratory lesions in these tissues.

Imported seafood: Some of the countries Australia imports from are known to harbour highly pathogenic parasites in their seafood products. Fresh and frozen imports are predominantly from Thailand, China and Vietnam where parasites such as *Clonorchis sinensis*, the Chinese liver fluke, and *Opisthorchis viverrini*, the south Asian liver fluke are common. The safety of these products for human consumption in this country relies on Australian legislation which is reflected in the latest imported food control order. At present no additional tests are applied to detect zoonotic parasites in seafood on entry to Australia (Imported Food Control Order 2019 in force 17 September 2019; https://www.legislation.gov.au/Details/F2019L01233). Australia relies on equivalency of the exporting countries testing procedures to provide documentary evidence of their adherence to internationally recognised food safety controls (Imported Food Control Order 2019 in force 17 September 2019; https://www.legislation.gov.au/Details/F2019L01233) such as parasite control detailed in the Codex Code of practice for fishery products (Codex Code of practice for fishery products; http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FFStandards%252FCXC2%252F2003%252FCX%253A%252F2003%252FCXP%253A%252F052e.pdf). Freezing and cooking are effective in killing the parasites.

Human movement: Australia is a popular destination for both tourists and migrants from both developed and developing countries. Seafood-borne parasites are also common in both developing and developed countries across the globe and, unlike many other parasites, are not limited to low income communities. Several seafood-borne parasites, such as *C. sinensis*, *Heterophyes heterophyes*, *Opisthorchis spp.* and *Dibothriocephalus spp.* reach adult stage in humans, producing thousands of eggs a day. Several species of other parasites from the same family, and some from the same genus, as the aforementioned zoonotic parasites are endemic in Australia. The possibility therefore exists that their intermediate hosts may be successfully exploited by exotic zoonotic species as part of their life cycle. Australia is also home to potential gastropod intermediate hosts such as native thiarid species and *Gabbia australis*, which may make establishment of a parasite life cycle viable. Currently there is no requirement to screen travellers and immigrants for infection with these parasites at the time of the entry to the country and this is seen as a viable entry point.

The unknowns

Transmission patterns and ecology: The detailed life cycle of zoonotic nematodes, in particular developmental stages which occur between the egg of the parasite and the infective stage (third stage larva, L3) are not fully known in Australia. These life stages may take place in a broad range of invertebrates and smaller fish (Figure 2). Also little understood are the major drivers that influence parasite distribution in these animals. Understanding life cycles of these parasites is central to determining the potential infection source, to designing effective educational campaigns for various stakeholders and to developing practical control and prevention programs.
Another emerging concern is understanding the effects of climate change on fish-parasite systems. Climate change unquestionably alters the prerequisites for parasite transfer, most likely to favour zoonotic parasites. Increased water temperature, for example, usually results in parasites spreading more rapidly and higher infection rates in fish.

Clinical knowledge and diagnostics: Correct diagnosis relies on effective communication between the patient, the clinician and the laboratory personnel and their awareness of pathogens. In Australia accurate diagnosis of seafood-borne parasitic diseases is very challenging. In the author’s experience one of the main reasons for this is lack of awareness and low diagnostic suspicion regarding seafood-borne parasites among key stakeholders, clinicians and laboratory personnel. As an example, when a patient presents with acute gastrointestinal signs inclusive of a history of raw seafood consumption a differential diagnosis of seafood-borne parasitism is not routinely considered. Anisakids/raphidascarids nematodes are also initiators of allergic reactions in humans. In many countries it has been confirmed that many previously diagnosed seafood allergies are an allergy to the parasite rather than the seafood itself. Although fish and shellfish allergy is one of the most common allergy in adulthood, in Australia nothing is known of the percentage of the ‘allergic population’ that may be attributable to parasites of seafood.

Should clinicians consider seafood-borne parasitic diseases in their action plans, there is no standard diagnostic test for these parasites in Australia. Reliance is being placed on the diagnostic tests developed overseas which, quite often, are not suitable to diagnose cases acquired in Australia and have variable/low specificity and sensitivity. For example, the recent human cases in Western Australia and Queensland were diagnosed as *Gnathostoma* based on a serologic test developed in Thailand, specificity and sensitivity of this test being uncertain. Interestingly, there is no report of this parasite in any Australian fish and none of the previous reports of *Gnathostoma* in other animals (such as cat, dog, bandicoot, quoll and pig) provides a
A closely related parasite from the same family as *Gnathostoma* is *Echinocephalus* and both share a high degree of morphological similarity. Parasites belonging to the genus *Echinocephalus* are commonly found in Australian fish and molluscs but has not been considered as zoonotic in government risk assessment studies due to lack of reports of human illness. In some Asian countries, following successful experimental infection of kittens and primates with *Echinocephalus* larvae, and due to popularity of consuming raw seafood, this parasite has been recommended to be considered of public health importance due to lack of reports of human illness. In some Asian countries, following successful experimental infection of kittens and primates with *Echinocephalus* larvae, and due to popularity of consuming raw seafood, this parasite has been recommended to be considered of public health importance due to lack of reports of human illness. Although presence of *Gnathostoma* spp. cannot be fully ruled out in Australia because there has never been a comprehensive study on seafood-borne parasites in this country, the fundamental concern is in the utilisation of serologic tests developed overseas to accurately identify Australian endemic parasites. Hence, it is likely that seafood-borne parasitic diseases are an underrecognised/underdiagnosed and underreported condition in Australia.

**Recommendations**

As long as there is awareness, prevention of infection with seafood-borne parasites is simple and easily doable (Figure 3). As rightly recommended by the National Health and Medical Research Council, Australia’s leading health research body, Australians should eat more fish. However, this recommendation should be supported by a strong body of research and education toward seafood safety to ensure the risk due to parasites is minimal.

**Research:** Some of the urgent areas for research would be:

- Determining the occurrence and the life cycle (including transmission patterns) of seafood-borne parasites in Australian marine and freshwater systems.
- Developing reliable tests specifically to detect infections both in humans and infected seafood for parasites prevalent in Australia.
- Determining factors contributing to the low awareness of these important parasites and diseases caused across multiple stakeholders.

**Translation of research outcomes including education:** In Australia the gap between scientists and other stakeholders involved in seafood safety is significant. In the recent risk assessments the frequent presence of zoonotic parasites reported in commonly wild caught fish has been fully overlooked and the number of human infections in the country has been significantly underestimated with two documented reports only! A preliminary study also shows that the lack of awareness among medical doctors in Australia is significant. Another challenge is the increasing decline in expertise in parasitology and reduction in the parasitology curriculum in Australian medical and veterinary schools. Research based education, communication and decision making of all stakeholders is central to seafood safety in this country. This includes fisher people, fish farmers, importers of seafood product, chefs, aquatic veterinarians, public health experts, clinicians, diagnostic laboratory staff, general and at-risk communities, and jurisdictional and federal agencies.

Figure 3. Some suggested methods for preventing infection with seafood-borne parasites, including anisakids/raphidascarids that are commonly found in Australian wild caught marine fish. Similar protocol and fact sheet can be easily disseminated to raise awareness. Note the temperature and duration suggested in the image is subject to the size of the fish fillet.
Conflicts of interest
The author declares no conflicts of interest.

Acknowledgement
The author thanks Ms Michelle Williams (Charles Sturt University), who assisted with the design of Figure 3.

References

Biography
Associate Professor Shokoofeh Shamsi has completed a Master degree in Medical Sciences and a PhD in Veterinary Sciences. She is currently based at School of Animal and Veterinary Sciences, Charles Sturt University. She leads a research group mainly working on seafood safety, aquatic animal health and diseases, wildlife parasitology, and emerging zoonotic diseases.
**Brucella: not your ‘typical’ intracellular pathogen**

Currently the genus *Brucella* consists of a group of bacteria that are genetically monospecific yet phenotypically diverse, and a recent genetic and phenotypic divergent group known as ‘atypical’ *Brucellae*. The host range is extremely varied and includes mammals, including humans, terrestrial animals and marine mammals, but now extends to reptiles and amphibians. Almost all *Brucella* species are zoonotic. The disease collectively termed *Brucellosis* leads to abortion and reproductive disease in animals, whereas human infection presents as a non-specific undulating fever accompanied by general malaise, chills, joint pain, muscle aches, genitourinary disease and adverse pregnancy outcomes. These Gram-negative coccobacilli invade and replicate in the host macrophages where they can limit the effects of the host immune system and antibiotic treatment. Due to the phenotypic and genotypic diversity and close relationship with *Ochrobactrum* species, the genus *Brucella* presents challenges for accurate identification and recognition of new species.

The disease, *Brucellosis*, affects animals and humans, causing abortions and reproductive disease in animals, and is known as undulating fever, Malta fever or Mediterranean fever in humans. **The causative agent, *Brucella*, are facultative intracellular, small Gram-negative coccobacilli (Figure 1) 0.5–0.7 μm x 0.5–1.5 μm that survive in the phagocytic cells of the infected host and were first identified in 1887. Transmission occurs through direct contact with infected material via mucus membranes, broken skin, ingestion or inhalation. Animals tend to recover but can continue to shed the bacterium into the environment, which makes control of *Brucellosis* challenging.**

**Brucella** species tend to be host specific but can infect other hosts although the disease is usually self-limiting in a non-primary host. Currently, there are 12 species of *Brucella* and can be divided into classical *Brucellae* (*Brucella melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis* and *B. neotomae*), marine mammal (*B. ceti* and *B. pinnipedialis*) and recently identified species (*B. inopinata*, *B. microti*, *B. paponis* and *B. vulpis*). There are also additional strains, awaiting genus affiliation, isolated from human and animal sources. A selection of recent species exhibits different phenotypic traits and greater genetic diversity than those in the classical group and are designated ‘atypical’ *Brucellae* (*B. microti*, *B. inopinata* and *B. vulpis*).

Most *Brucella* species pose a significant zoonotic threat to humans most notably *B. melitensis*, *B. suis*, *B. abortus* and *B. canis*. Other *Brucella* species including those that affect marine mammals are also zoonotic. To date only *B. ovis*, which causes reproductive failure and abortion in sheep, is not zoonotic.

The taxonomy of *Brucella* presents challenges. Traditionally, new species were named according to the host from which they were isolated, and biovars were assigned to reflect the diverse range of phenotypes within some species. Classical *Brucella* species are 90% homologous, which means that the genus is monospecific according to the designation of species as having greater than 70% homology using DNA-DNA hybridisation. In this case *B. melitensis* is the only species, and the rest are biovars. However, a consensus
held by the scientific community has seen the traditional species names retained irrespective of the traditional taxonomic conventions. Interestingly, application of molecular typing methods such as pulsed field gel electrophoresis, infrequent restriction site polymerase chain reaction, restriction fragment length polymorphisms, insertion sequence site testing, multilocus sequence analysis, variable number tandem repeat analysis and genome sequencing show clustering of genotypes that supports the classical designation of species and biovars.

Greater genetic diversity exists among the atypical Brucella clade than in the classical clade (Figure 2). Atypical Brucellae diversity has been attributed to the ability of these basal species to exchange DNA with each other and with other microbes in the environment using horizontal gene transfer. The atypical group includes...
designated species (as mentioned earlier) as well as candidate strains like BO2 from a human, LT605586 from a bluespotted ribbontail ray and NF2653 from a rodent. Recently, atypical Brucella species have been isolated from amphibians. Some Brucella isolates obtained from amphibians are most closely related to B. inopinata BO1 and Brucella-like BO2 strains based on whole genome analysis. Given their similarity to BO isolates, for which the animal reservoir has not yet been identified, amphibians might represent a possible source of these strains. Recently, Brucella from domestic marsh frogs in France were found to be more similar B. microti and not B. inopinata.

Isolation of frog Brucella sp. from Africa, Europe, Australia and America suggests that they may be widespread and highlight a need for a broader assessment of the presence of Brucella in amphibians worldwide. A major aspect of Brucella virulence is their capacity to replicate inside macrophages and escape the host immune system. Recently, in vitro and in vivo infection experiments with amphibian Brucella isolates found that isolates were able to invade and even multiply intracellularly in macrophages and survive in the murine host for up to 12 weeks. Given the lack of definitive evidence and their proximity with strains associated with human disease, isolates from amphibians should be considered as potential zoonotic pathogens.

**Diagnoses**

Accurate identification of pathogens is essential for establishing dependable diagnosis, choosing a treatment, and understanding the source of infection. Conventional identification of Brucella is based on modified acid fast staining and phenotypic methods including phage typing and serology that differentiate the species and biotypes. These tests are usually done in a specialist laboratory because of the types of tests conducted and the biohazard of working with the organism. B. melitensis can be identified using matrix-assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectroscopy; however, further differentiation of species is not available using this technology without additional curation of the reference database. It is important to differentiate Brucella and Ochrobactrum species (close genetic relative within the family Brucellaceae, a genus largely consisting of environmental bacteria occasionally infecting humans) as Brucella has a higher biosafety risk to hospital and laboratory staff and can have different treatment strategies. Due to their similarity, some routine commercial identification systems can mis-identify the two organisms as B. melitensis and O. intermedium have a 98.8% similarity according to the rRNA gene sequence. Atypical Brucellaceae further complicate laboratory identification as most members, including amphibian isolates, are motile. Amphibian isolates also exhibit variant lipopolysaccharides (LPS), phage lysis, serum agglutination and dye sensitivities compared to classical Brucella and are often misidentified as Ochrobactrum. Although, when the Brucella reference database is available, MALDI-TOF assays can correctly identify them as Brucella. The differences in LPS of atypical and classical Brucella could result in serological diagnostics being impaired especially for human infections.

Until recently the genus Brucella was considered to represent a genetically homogeneous group of bacteria associated with mammalian hosts. Recently, the situation has become more complex with a rapid increase in the number of novel, genetically divergent, Brucella being isolated from cold-blooded hosts. The zoonotic potential and pathogenicity of these Brucella sp. strains remains unknown. Further studies are required to gain insights on the bacterial carriage and characterisation of these isolates to understand their role in the evolution of the species from being soil bacteria that are characterised by motility and a broad metabolic activity to becoming highly virulent but host-specific clonal pathogens.

**Conflicts of interest**

The authors declare no conflicts of interest.

**Acknowledgements**

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Glanders: re-emergence of an ancient zoonosis

Both legal and illegal, mainly for performance purposes, has enhanced the risk of global spread of glanders in the Middle East and elsewhere. Ever since the First World War, the glanders bacillus has been recognised as a potential biological warfare agent.

The organism
Glanders is an OIE (World Organisation for Animal Health) listed notifiable disease caused by *Burkholderia mallei*, a Gram-negative, non-motile and non-spore-forming bacterium. Previously known as *Pseudomonas mallei*, it is genetically closely related to the agent of melioidosis, *Burkholderia pseudomallei*. It is an obligate pathogen of domestic equids. Glanders is one of the oldest long recognised as a very important zoonotic disease of humans. The incubation period of glanders varies from a few days to many months according to the route and level of exposure and infection. The organism is highly pathogenic, with a case fatality rate of up to 70% in untreated cases.

Glanders, although known to be endemic in certain regions/countries of the Old and New Worlds for centuries, had been largely overlooked as a threat to equine and human health until the disease re-emerged in the Middle East in 2004. The exponential growth in international horse movements, both legal and illegal, mainly for performance purposes, has enhanced the risk of global spread of glanders in the Middle East and elsewhere. Ever since the First World War, the glanders bacillus has been recognised as a potential biological warfare agent.

**Biographies**

**Dr Anthony Keyburn** is Senior Scientist at the AAHL. His research interests are on brucellosis and veterinary tuberculosis pathogenesis and diagnostic and vaccine development.

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25. (2018) *Burkholderia pseudomallei*, a Gram-negative, non-motile and non-spore-forming bacterium. Previously known as *Pseudomonas mallei*, it is genetically closely related to the agent of melioidosis, *Burkholderia pseudomallei*. It is an obligate pathogen of domestic equids. Glanders is one of the oldest recorded diseases of horses dating back to Aristotle (350 BC), and long recognised as a very important zoonotic disease of humans. The incubation period of glanders varies from a few days to many months according to the route and level of exposure and infection.
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**In Focus**

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infection with
This is a hypersensitivity skin test which does not distinguish
include the Mallein test (preferably the intradermo-palpebral test).
infections are sub-clinical or latent. Other tests resorted to can
include strangles (Streptococcus equi equi), a common disease of
horses, melioidosis (B. pseudomallei), epizootic lymphangitis
(Histoplasma capsulatum var farciminosum), ulcerative lym-
phangiitis (Corynebacterium pseudotuberculosis) and sporotri-
chosis (Sporotrichum schenckii). Acute disease occurs mainly in donkeys; glanders in horses can be
acute, but more often chronic or latent. Humans are susceptible
and if untreated, infection in man is frequently fatal. Small rumin-
ants and dromedary camels kept in close contact with infected
horses can contract the disease, as can carnivores by eating infected or contaminated meat.

Diagnosis
Glanders presents diagnostic challenges and diagnosis cannot be
based on clinical signs alone. Isolation of B. mallei from lesions and
exudates is the ‘gold’ standard; difficulties arise however when infections are sub-clinical or latent. Other tests resorted to can
include the Mallein test (preferably the intradermo-palpebral test).
This is a hypersensitivity skin test which does not distinguish
infection with B. mallei from B. pseudomallei.

The complement fixation test (CFT), the OIE recommended se-
rological test for international trade, is complex to perform,
difficult to standardise and can result in anti-complementary reac-
tions with some sera. Its sensitivity and specificity can vary depend-
ing on the antigen and methodology used. Newer more sensitive
and specific diagnostic tests e.g. the polymerase chain reaction test,
competitive enzyme-linked immunosorbent assay (C-ELISA) and
the Western immunoblot test have been developed to overcome
the limitations of traditional diagnostic tests. However, none of
these tests have yet been validated by the OIE for use in interna-
tional trade.

Transmission
Glanders is usually introduced to a free area by an asymptomatic carrier animal. Spread can occur by direct or indirect contact with
an infected animal. The disease is frequently contracted by the
ingestion of food or water from communal troughs contaminated
by nasal discharges or pus from skin lesions. Spread by inhalation
can also occur. The cutaneous form of glanders is spread by
contaminated saddlery, harness or grooming equipment. Latent
infection can be reactivated by stress, for example, travel, poor
husbandry or over-crowding.

Disease in humans
Naturally occurring disease occurs infrequently in humans and
usually results from occupational exposure involving close and
prolonged contact with an infected equid(s). People most at risk
include veterinarians, grooms and abattoir workers. It is now
generally accepted that glanders caused the death in 1793 of
Charles Vial de St Bel, inaugural Professor of the recently estab-
lished London Veterinary College, although the zoonotic potential
of glanders was not actually recognised until the beginning of the
19th century. Deaths in laboratory workers have also been
reported, including European scientists studying glanders after
the initial isolation of the aetiologic agent in 1882, and military
research microbiologists in the USA.

B. mallei was used as a biological warfare agent against animals in
Europe, Russia and the USA during the First World War. In the USA,
cultures were grown by a German agent in a basement laboratory in
Washington, DC. The microbes were suspended in liquid in test
tubes, and dockworkers recruited by the Germans went among
mules and horses assembled for shipment to the Allied forces in
Europe and jabbed animals with needles that had been dipped into
the microbial cultures.

Glanders was classified as a Category B biothreat agent by the Centres
for Disease Control and Prevention in the USA and all potentially
infected or contaminated material must be handled in a laboratory
with appropriate biosafety and biosecurity controls.

Geographic distribution and re-emergence
Glanders was once widespread globally primarily due to the move-
ment of horses for war, transportation and agriculture. Throughout
history, when horse-mounted troops invaded new territories,
glanders almost invariably accompanied them. Mandatory na-
tional test and slaughter control programs in the early to mid-20th
century eradicated the disease from many countries, including the
USA, Canada and Western Europe. Since then, these countries have

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maintained their glanders-free status by strict import policies and border controls1,7,8.

Glanders still persists in parts of Eastern Europe, the Middle East, Asia, Africa, the Indian subcontinent, and Brazil in South America1,3. Over the past 15 years glanders has been reported with increasing frequency from the Middle East (United Arab Emirates, 2004; Iran, 2007; Kuwait, 2009 and 2019; Bahrain, 2010; Lebanon, 2011) and is considered a re-emerging disease in that region1,15,16. Cases have been reported from Kuwait as recently as July 201917 and most recently in Turkey in late 2019. Movement of horses from Syria appears to have played a prominent role in the disease’s re-emergence16,18,19.

Glanders has also made an unwelcome reappearance in Western Europe. A 2009 report described a horse imported into Germany from Brazil in 2006 with certification of no known exposure to the disease and of negative CFT status. It became ill after arrival and the animal was subsequently diagnosed as having glanders15,20. In 2015, another case; and this time of latent or occult glanders, was identified in a German horse where the source of infection was not identified21. Confirmation of a case of glanders has a substantial economic impact in non-endemic areas. There is no effective long-term treatment or any vaccine against the disease. Horses eventually die. A test and slaughter program must be introduced if a country wishes to regain its free status2. Diseased animals and animals that test positive must be killed, severe restrictions on horse movement imposed within the country and the international trade in equids and their products suspended for an extended period.

What factors are contributing to the renaissance of this ancient disease?

- Foremost is the exponential growth in international movement of horses for equestrian sports or racing purposes, particularly legal and illegal movements between Middle Eastern countries,8,15,16.
- Inadequate regulation, biosecurity and supervision of international horse movements including failure of countries to harmonise their national regulations for importation with OIE disease specific recommendations15.
- Regional conflicts can disrupt national government control programs1.
- B. mallei can evade the host’s immune response and result in latent infections and the potential for the introduction of infection into glanders-free countries9.
- Absence of an OIE validated diagnostic serological test for glanders. The current OIE approved CFT test for international trade can give rise to false positive and false negative results. Unreliable diagnostic tests compromise the efficacy of disease control programs1.
- Lack of awareness of the clinical signs of glanders may result in an incorrect or delayed diagnosis and failure to detect or report an outbreak. Reduced commercial use of horses has led to diminished veterinary awareness of glanders, its clinical signs, epidemiology and diagnostic methods7,8.
- Disease investigations carried out by private veterinarians have hampered government authorities and prevented timely and appropriate action10.
- Failure to report the disease – economic and cultural circumstances may hinder the culling of asymptomatically infected animals; fear of restrictions and the absence of adequate compensation may also deter official reporting14,7,19.

An Australian perspective

Glanders has never gained a foothold in Australia. Australia was remote from Europe (and its major wars and disturbances) and had no land borders. It was not an attractive target for European invaders until the British came by sea to establish the first penal colony in 1788 accompanied by a few horses sourced from South Africa22. Luckily these and other horses imported in the early wild colonial days did not introduce glanders. Freedom from significant equine diseases such glanders provided an important trade advantage for the export of Australian horses to the Indian market as army remounts23.

Nevertheless, in November 1891, swift and efficient action by New South Wales (NSW) quarantine authorities prevented disaster. Glanders was detected by a government veterinarian in circus horses imported from the USA. Fortunately, they were still held in quarantine. Their only Australian contacts were horses recently recruited to the circus. The two imported clinical cases and their Australian contacts were destroyed. The imported horses were then transferred to Shark Island in Port Jackson where four more animals developed the disease. The remaining circus horses were shipped back to San Francisco in February 1892. Their export occurred despite considerable opposition from certain NSW veterinary practitioners who thought the rest of the American circus horses could be safely admitted to Australia because the diseased animals had been killed. Unlike the recent experience in the Middle East16, the strong stand taken by the government authorities prevailed24.

Subsequently, Australia has maintained strict border controls and maintained its glanders free status. An import risk analysis covering inter alia the biosecurity risk posed by glanders in imported horses was conducted in 2010 and reviewed in 201325,26. Australia only imports horses directly from countries in which glanders does not occur. To be eligible for import, horses must have been resident for at least 6 months prior to shipment or since birth in an approved country free of glanders.

In the unlikely event that glanders did ever manage to penetrate our borders again, Australia is well prepared for its eradication with a response plan and a government/industry disease control cost sharing formula agreed in advance of any outbreak27.
Future research

To improve global control of glanders while facilitating future international horse movements, the International Horse Sports Confederation and the OIE have supported projects to identify and validate improved diagnostic tests. More sensitive tests will allow more accurate certification of the disease freedom of individual animals and reduce the risk of introduction of glanders to new areas by the international movement of infected carriers. More specific tests will also reduce false positive results that lead to unnecessary and expensive obstacles to trade.

Conflicts of interest

The author declares no conflicts of interest.

Acknowledgements

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Biography

Dr Patricia Ellis is a veterinarian with global horse industry regulatory and clinical experience. Her interests include safe international movement of horses, import risk analysis, biosecurity and emergency animal disease management.
COVID-19: a novel zoonotic disease caused by a coronavirus from China: what we know and what we don’t

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At the end of December, 2019, a new disease of unknown aetiology appeared in Wuhan, China. It was quickly identified as a novel betacoronavirus, and related to SARS-CoV and a number of other bat-borne SARS-like coronaviruses. The virus rapidly spread to all provinces in China, as well as a number of countries overseas, and was declared a Public Health Emergency of International Concern by the Director-General of the World Health Organization on 30 January 2020. This paper describes the evolution of the outbreak, and the known properties of the novel virus, SARS-CoV-2 and the clinical disease it causes, COVID-19, and comments on some of the important gaps in our knowledge of the virus and the disease it causes. The virus is the third zoonotic coronavirus, after SARS-CoV and MERS-CoV, but appears to be the only one with pandemic potential.

An outbreak of cases of pneumonia of unknown aetiology in the city of Wuhan in Hubei Province, China, was announced and notified to the World Health Organization (WHO) by the Wuhan Municipal Health Commission on 31 December 20191. The outbreak was linked epidemiologically to the Hua Nan seafood and wet animal wholesale market in Wuhan, and the market was subsequently closed on 1 January 2020. A week later, on 7 January, the isolation of a previously unknown betacoronavirus was reported as the aetiological agent.

Wuhan is a city of 11 million inhabitants and is a major transport hub, and over the ensuing weeks the virus spread to other provinces in China and later to an increasing number of other countries. This spread prompted the WHO Director-General to establish an Emergency Committee (EC) under the International Health Regulations (IHR). The EC recommended that the outbreak constituted a public health emergency of international concern at its meeting on 30 January2. In so doing, the Committee believed that it was still possible to interrupt virus spread, provided that countries put in place strong measures to detect disease early, isolate and treat cases, trace contacts, and promote social distancing measures commensurate with the risk.

The city of Wuhan was placed in quarantine by the Chinese Government on 23 January, stopping all rail, road and air transport out of the city. The quarantine was subsequently extended to a further 17 cities in Hubei Province, affecting over 57 million people, which was particularly challenging as it came two days before the Chinese New Year, the most important festival in the country, and traditionally the peak traveling season. Since then there have been increasing measures to control and manage the epidemic within China, and the introductions of numerous travel restrictions by other countries that either have had cases or are trying to prevent entry.

Early events in determining the identity and origin of the novel coronavirus

Whole virus genome sequences were obtained either directly from patient samples or from cultured viruses from a number of patients hospitalised with pneumonia in Wuhan, showing that the aetiological agent was a betacoronavirus belonging to a new clade in
subgenus Sarbecovirus in the Orthocoronavirinae subfamily. Phylogenetic studies of the new virus showed it shared about 79% nucleotide homology with SARS-CoV, as well as to two SARS-like coronaviruses isolated from Chinese horseshoe bats (Rhinolophus sinicus) in Zhoushan, with which it shared 89% nucleotide homology, and to a third SARS-like coronavirus from an Intermediate horseshoe bat (R. affinis), with which it shared 96% nucleotide homology. Based on established practice, the new virus was named SARS-CoV-2 by the Coronavirus Study Group of the International Committee for the Taxonomy of Viruses, and the disease it causes as COVID-19 by WHO.

How this virus moved from animal to human populations is yet to be determined. The outbreak clearly began epidemiologically at the Wuhan market, and a number of environmental samples from around the live animal section of the market were subsequently found to be positive for SARS-CoV-2, but based on current evidence, it may not have actually emerged in the market. The earliest recognised case of infection with SARS-CoV-2 was an elderly man who developed symptoms on 1 December 2019. None of his family members became infected, and the source of his infection remains unknown. Furthermore, 14 of the first 41 cases had no contact with the seafood market. In another report, five of the first seven cases of COVID-19 had no link to the seafood market.

Thus, it seems very likely that the virus was amplified in the market, but the market might not have been the site of origin nor the only source of the outbreak. A recent phylo-epidemiological study has suggested that the virus was circulating but unrecognised in November, and was imported to the seafood market from elsewhere, where it subsequently was amplified.

Angiotensin-converting enzyme II (ACE2) was known to be the cell receptor for SARS-CoV, and also for some SARS-like bat coronaviruses. Sequence studies found that the receptor-binding domain of the SARS-CoV-2 virus was sufficiently similar to that of SARS-CoV to indicate it could efficiently use the human ACE2 receptor for entry to human cells. Infectivity experiments were undertaken with HeLa cells expressing or not expressing ACE2 from humans, bats, civets, pigs and mice, and the results confirmed that SARS-CoV-2 virus was able to use entry receptors on all ACE2-expressing cells other than mice. Molecular modelling has indicated that the binding affinity of SARS-CoV-2 to ACE-2 may be even higher than that of SARS-CoV and it may therefore be more efficient at infecting human cells. Evidence from the sequence analyses clearly indicates that the reservoir host of the virus was a bat, probably a Chinese or Intermediate horseshoe bat, and it is probable that, like SARS-CoV, an intermediate host was the source of the outbreak. To ensure that future cross-species transmission events of this new virus don’t occur again in the future, it is important to identify the reservoir and intermediate wildlife hosts. The closest known wildlife sequence to SARS-CoV-2 remains the sequence from the virus isolated from an Intermediate horseshoe bat, but there were significant differences in the receptor-binding domain between the two viruses. Malayan pangolins (Manis javanica) have been suggested as potential intermediate hosts, and SARS-like viruses have been identified in pangolins seized in anti-smuggling operations in southern China, but they only shared about 85–92% homology with SARS-CoV-2. No other possible intermediate wildlife host has been proposed at this time.

Next generation sequencing (NGS) was carried out on lung lavage samples from up to 17 patients between 24–30 December 2019 that would have demonstrated the presence of a SARS-related coronavirus, but this information was not widely available until a sequence was reported on 12 January 2020. Interestingly, this was the first time that NGS had alerted the world to a new zoonotic virus before the virus had been isolated, and it suggests that a new procedure for reporting outbreaks based on NGS rather than pathogen isolation and identification needs to be considered.

The resulting information flow was a great improvement over the experience with SARS-CoV in 2003, but it is important to continue improving this as the lack of the earliest possible information about the sequences slowed down the development of diagnostics and preparedness capacity. This lack of early information may have extended to case notifications, as no cases were reported between 1 and 17 January 2020, but modelling suggested there may have been over 450 cases unreported in that time, and indeed a number of such cases were subsequently confirmed retrospectively.

Transmission

Human-to-human transmission of SARS-CoV-2 has been widely shown in health care, community and family settings. The dominant mode of transmission is from the respiratory tract via droplets or indirectly via fomites, and to a lesser extent via aerosols. In addition, as SARS-CoV and MERS-CoV can infect the human gastrointestinal tract, it has been suggested that faecal-oral spread may occur for SARS-CoV-2. The reproduction number (Ro) is generally thought to be between 2.0 and 2.8, although higher reproduction numbers have been suggested in some reports. The mean incubation time appears to be between 4.75 and 7 days, ranging from 3 days to an upper limit of around 11–14 days. There is increasing knowledge about the virus load. In one study of symptomatic patients, higher viral loads were detected soon after symptom onset, with the viral loads higher in the nose than in the throat. In a single asymptomatic patient, the viral load was similar to the symptomatic patients. In a second and more detailed study, the virus load was investigated over

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consecutive days in two patients from the time of their hospitalisation, with serial samples throat swabs, sputum, urine and stools. The viral loads peaked around 5–6 days after symptom onset, with $10^4$ to $10^5$ copies/mL. The authors also studied the viral loads in throat swabs, sputum and stool samples in other patients, and found viral loads were as high as $10^1$ copies/mL in throat samples, but with a median of $7.99 \times 10^4$, and $7.52 \times 10^5$ in sputum. In addition, virus was detected by RT-PCR in stools from 9 of 17 confirmed cases, but at titres lower than in respiratory samples. Several studies have indicated that transmission may occur during the incubation period and from asymptomatic or very mild infections.

There are a number of important questions still to be answered about the transmission dynamics. These include information about the infectivity during the incubation period; the length of time and virus load during incubation and during the symptomatic period of virus shedding; the incidence and infectiousness of asymptomatic cases, the risk of vertical transmission from mother to fetus, and other modes of transmission, such as from faeces, saliva and urine. There is some evidence that virus can be isolated from saliva and other modes of transmission, such as from faeces and urine. The number of mild or asymptomatic cases has not been determined and relatively few cases have been recorded, but it is probable that current figures only see the tip of the iceberg, and many cases remain undiagnosed. They pose the greatest threat for increased virus spread. Information is also needed on the stability of the virus in the environment to better determine transmission risks, and especially the survival of the virus in aerosols and on hard surfaces under different conditions of temperature and humidity.

**Clinical features**

It has become clear that asymptomatic infections and minimally symptomatic infections occur with this virus. Exactly how frequently is not yet known as that requires serological studies that have yet to be undertaken. The initial reports of the illness were heavily biased to more severe and hospitalised cases in China, and as the number of confirmed cases has increased within China and elsewhere, a clearer picture has emerged. The commonest clinical features are fever plus a respiratory illness, and studies have reported fever in 80–99% of cases, dry cough in 48–76% of cases, fatigue or myalgia in 44–70% of cases, and dyspnoea in 30–55% of cases. Other relatively frequent manifestations include anorexia and productive cough, and less frequently, headache, diarrhoea, nausea, dizziness and vomiting. Severe illness and death are more likely to occur in older individuals, and possibly in those with pre-existing clinical illness such as diabetes, cardiovascular disease and malignancies.

The case fatality rates have varied depending on the population affected. Initial estimates that were based on severely ill patients were high, but more recent estimates are around 2.3% on average, but significantly higher in the elderly and particularly those aged 80 years and over. As many milder or asymptomatic infections are likely to have been missed, the mortality rate is expected to be lower than published figures as more information becomes available. Disease in paediatric patients appears to be rare, and when it occurs, very mild, but the role of children in transmission remains unknown.

More extensive and detailed studies are needed to understand the full spectrum of illness caused by this virus, the pathogenesis of disease, especially the lung disease, and the longer-term morbidity in survivors. The possibility of persistent and recurring infections, especially in immunocompromised patients, is unknown; we still have very limited data on the disease in pregnancy and the potential for infection of the fetus; and we don’t understand the effect of past exposure to other coronaviruses on modifying disease severity.

**Diagnostic tests**

Clinical diagnosis has largely been based on clinical and exposure history, and laboratory and chest imaging findings. The laboratory findings will vary with the severity of disease, but a low lymphocyte count is common and persisting low counts is associated with poorer outcomes. Testing for other respiratory pathogens should be undertaken to exclude viral and bacterial co-infections.

Detection of the virus has been based on PCR, with various assays directed particularly at the envelope (E), RdRp, spike protein (S) and nucleocapsid (N) genes. In-house assays are in use in a number of Public Health Laboratory Network (PHLN) member laboratories, with continual ongoing evaluation. In addition, there are several commercially available assays with claimed capacity for detection of SARS-CoV-2 that are also being evaluated. Virus can be found in the upper respiratory tract in nearly all patients beginning at or just before the onset of clinical illness. The preferred samples are combined nasopharyngeal and oropharyngeal swabs and if they have a productive cough, also sputum. In patients with lower respiratory tract infections, virus may be detectable in sputum or bronchoalveolar lavage samples even if undetectable in the upper...
respiratory tract. Virus can be cultured relatively easily in a number of cell lines, including Vero cells, but requires a PC3 containment laboratory. Virus identification can be confirmed by sequencing if necessary, and a large number of partial and whole genome sequences are available via GISAID and GenBank. PCR tests have become increasingly available since about 25 January, but due to the rapid spread in China and the huge number of cases access to timely and accurate testing has been problematic, especially in the early stages, making it difficult to ascertain the true extent of virus infections and illness.

Detailed PHLN recommendations for testing and for laboratory biosafety requirements within Australia are available in the Virology Appendix of the Coronavirus Disease 2019 (COVID-19): CDNA National Guidelines for Public Health Units. However, tests are still being developed and evaluated, and we cannot yet confidently identify the best targets for PCR. Continual re-evaluation is required to identify any genetic drift that may affect test sensitivity. We also need to be able to transfer the tests onto platforms that can be delivered outside major laboratories, in resource poor settings, near to the patient, and quickly. The lack of serological assays hampers our ability to understand the true epidemiology of this virus and its impact, and to identify PCR-negative infections.

**Therapeutics and vaccines**

There are no proven or registered therapeutics or vaccines for COVID-19 infection at this time. Treatment is largely supportive, though a number of therapeutics are under investigation with some undergoing clinical trials in China and elsewhere. Of particular interest currently are the HIV protease inhibitor combination lopinavir/ritonavir and a new broad-spectrum antiviral agent called remdesivir, which has shown promising activity against MERS-CoV in animal models. Combinations of these with interferon-β and/or ribavirin are being considered, while other groups are looking at other antivirals, convalescent plasma, and monoclonal antibodies. Work on vaccines is also well underway, although it is unlikely that a vaccine will be available for at least 18 months. The Coalition for Epidemic Preparedness and Innovations (CEPI) is currently funding four vaccine initiatives in collaboration with the WHO. One of these is at The University of Queensland where Prof Paul Young, and Drs Keith Chappell and Dan Watterson are using their novel and exciting ‘molecular clamp’ technology to develop a vaccine. This work is being carried out in collaboration with CSIRO’s vaccine manufacturing plant in Clayton, and also partly funded by CSL Ltd. This Australian collaboration is hoping to have the vaccine ready for use before the end of 2021.

**Current status**

The international public health response to COVID-19 has largely been based on measures that have proved successful for many other outbreaks over past decades – rapid identification, management and isolation of cases; identification and follow up of contacts; quarantine measures; social distancing; infection prevention and control in health care settings; community containment; and transparent risk communication; as well as a 14-day quarantine period on travellers from China or who have had links to ongoing transmission sites or known cases. As the cruise liner in Japan. Australia’s response has been similar, and to date has been moderately successful in limiting the number of chains of transmission in NSW, Queensland and Victoria, whereas most cases elsewhere have been in returning travellers or their close contacts. Table 1 shows cumulative cases in Hubei, elsewhere in China, and in other countries over a weekly basis between 21 January and 9 March, and shows the rapid rise in cases in China, particularly in Hubei Province, followed by a slowing down over the past 2 weeks, and a continuing rise in cases outside China with more and more countries reporting cases. As of 9 March, 105 countries had reported cases, but only three countries accounted for nearly 75% of the cases; South Korea with 7382 cases, Italy with 7375 cases, and Iran with 6566, and cases have been reported from all countries.

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<th>Table 1. Cumulative cases of COVID-19 on a weekly basis from 21 January to 29 February 2020, for Hubei Province, for the rest of China, and for other countries.</th>
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<td><strong>Cumulative number of cases</strong></td>
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<tr>
<td>Cumulative number in Hubei</td>
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<td>Cumulative number in China (excluding Hubei)</td>
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<td>Cumulative number in other countries</td>
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<td>Number of countries reporting cases</td>
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continents except Antarctica. It has been suggested that up to half of COVID-19 cases exported from mainland China have remained undetected worldwide, and that 40% of travellers, largely asymptomatic, mild or pre-symptomatic travellers, were not detected at airports, potentially resulting in multiple chains of as yet undetected human-to-human transmission outside mainland China. Although WHO finally called the COVID-19 a pandemic on 11 March, in many respects it had already begun. This was presaged by the observation that more new cases were reported from the rest of the world than from China for the first time on 28 February, and in addition, secondary and tertiary chains of transmission have been reported in increasing numbers of countries. The Australian Government has already recognised this, and has instituted its emergency response plan and is now operating on the basis that the pandemic is here.

Final comments

SARS-CoV-2 is the seventh coronavirus known to infect humans, and the third zoonotic virus after SARS-CoV and MERS-CoV. Bats are the reservoir hosts of a number of additional novel coronaviruses, particularly Chinese horseshoe bats, and a number of these novel coronaviruses can efficiently use multiple orthologs of the SARS receptor, human ACE2, and replicate efficiently in primary human airway cells and achieve in vitro titres equivalent to epidemic strains of SARS-CoV. This indicates that other potential cross-species events could occur in the future. There is therefore a strong reason to ban unregulated wild animal sales in Chinese wet markets, particularly exotic species, both from a public health perspective and for ecological reasons. Such a ban would be difficult to instigate for cultural reasons, but China’s top legislative committee on 24 February 2020, passed a proposal to ban all trade and consumption of wild animals. If this is legislated as a permanent ban, it might help reduce the risk of another novel virus emerging from wildlife in China in the future.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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References


Guest Editors: Ipek Kurtböke and Ian Macreadie
Leila Valerie James was born at Rupanyup in western Victoria in 1927. She attended local schools and continued her education despite significant family hardship. After matriculating from Ballarat High School she achieved her dream of going to The University of Melbourne and in 1944 commenced her BSc while living in nearby Brunswick. At Easter she fell ill with pleurisy. After a local GP drained 3 pints of fluid from her pleural cavity, she was sent home to rest, devastated to leave the university and her new friends. The word ‘tuberculosis’ (TB) was never mentioned to her.

In 1945 she returned and completed first year BSc, beginning second year in 1946. In July a letter arrived, telling her ‘your bed is waiting for you at Greenvale Sanatorium’, a great shock as she had not been told she had TB. Sanatorium life was very restrictive, with many hours of rest timetabled, few activities available and minimal visiting hours at weekends. In spite of these constraints, Val read widely and developed a love of needlecraft that lasted through her busy life. This was prior to the availability of antibiotics to treat TB and people were regularly dying around her. Also, assessment testing was done only every three months, so release could take many months. In 1947 Val was given barbaric surgery, involving a 38 cm cut down her back to enable ribs to be removed to immobilise the upper part of the lung, all done under local anaesthetic (supposedly to prevent spread to the other lung). Streptomycin became available only in the late 1940s, but the three doses Val received were inadequate for treatment.

She returned to the University after discharge from Greenvale in January 1948 and continued her degree; however, in 1949 the infection had spread to the right lung, subsequently requiring readmission to Greenvale and an artificial pneumothorax was performed in 1950. After release, she completed her 2nd year BSc from home in 1951. Medical experts later believed that she had contracted TB many years earlier while sharing a ward in Ballarat Hospital with a TB patient. She later wrote of her experiences in Walking My Baby Back Home – My Journey with TB without Antibiotics, one of her many books.

In 1952 Valerie commenced life at Janet Clarke Hall and completed her BSc, then MSc and commenced PhD research under Professor Sydney Rubbo, working on urinary tract infections in people with spinal injury. She was a tutor, demonstrator and then Principal Tutor at The University of Melbourne, and also a resident tutor at Janet Clarke Hall for 5 years, completing her PhD in 1975. During these years she also worked as a consultant with Westminster Carpets.

In 1958, at a Trinity College party, Val met barrister Austin Asche, originally from the Northern Territory. They were married 3 months later and subsequently had two children, Harry and Wendy. Austin later became the first Victorian Judge of the Family Court of Australia.

In the early to mid-1980s, Val was the Director of the Clinical Department of Microbiology at the Queen Victoria Medical Centre – at this time an exceptional position for a woman and a scientist. As this was Monash University’s teaching hospital for obstetrics, gynaecology and paediatrics, Val also held the post of Senior Lecturer. Those who worked with her in the laboratories and taught classes for her, speak of her excellent work and her great ability to interact with medical colleagues, scientists and students.

In 1986 Austin Asche was made a Judge of the Northern Territory Supreme Court and the couple moved to Darwin. Val was immediately appointed Senior Research Officer in the Microbiology Unit at the Menzies School of Health Research and Sessional Microbiologist at Royal Darwin Hospital. This, her first opportunity to participate widely in research, was very important to her. She held these positions until 1994. Under her leadership, research covered many fields, particularly involving diseases prevalent in the Northern Territory, including melioidosis and sexually transmitted infections. Val authored more than 40 papers, including the first from the southern hemisphere to report on the isolation of Chlamydia pneumoniae. She and her team always took great care to consider the comfort and sensibilities of the indigenous members of the community.

Meanwhile Austin Asche was appointed as Chief Administrator of the Northern Territory and the couple moved into Government House. During this period Val became patron of at least 35 community organisations. She saw that her role was to support and encourage these varied organisations.

On retirement from her Menzies post she was awarded the Menzies School of Health Research Medallion for outstanding service to the School, and in 1994 was appointed to the
Governing Board of the Menzies School of Health Research. While the Menzies Research Institute was initially linked to Sydney University, Val was an important figure supporting the formation and running of the new Charles Darwin University. She became chairman of both Community Radio 8 TopFM and the Australian South East Asian Rehabilitation Foundation, which sends medical teams to Timor and Flores. In 2002 she chaired the Task Force on Illicit Drugs in the Northern Territory, commissioned by the Northern Territory Legislative Assembly.

Over her working life, Val has given great service to microbiology and to the Australian Society for Microbiology. She was Treasurer of the ASM National Council 1978–1984, then Membership Secretary 1984–1986. She edited six editions of Recent Advances in Microbiology, then became Chairman of the Northern Territory branch of the ASM. She also convened the Trust to raise $2m for scholarships for young microbiologists. Portions of her interviews are in the Golden Jubilee issue of Microbiology Australia.

Dr Valerie Asche was an extraordinary woman, participating deeply in many aspects of life, especially in the Northern Territory. Scientist, community leader, craftswoman and friend to many. Her awards include: Distinguished Service Award Australian Society of Microbiology 1991; Dame of the Order of St John of Jerusalem 1993; Women’s Achievement Award for Outstanding Contribution to Northern Territory 1998; Distinguished Service Award of Australia 2000; Senior Australian of Year 2000; and Member of the Order of Australia 2001.

An updated view on bacterial glycogen structure

**Liang Wang and Michael J Wise**

The authors advise that in Figure 1 of their published article (Microbiology Australia, Volume 40, Issue 4, pages 195–199, doi:10.1071/MA19056) they linked GlgE directly with branched (glucosyl units)$_n$. In fact, GlgE should work together with GlgB to synthesise glycogen. In addition, although Rv3032 was initially postulated to have a possible role in glycogen metabolism, recent study has shown no detectable evidence to support this postulation. The authors apologise for this error and state that this does not change the scientific conclusions of the article in any way. The correct Figure 1 is shown below.

![Glycogen Metabolism Pathway](image)

**Figure 1.** Schematic illustration of classical and non-classical glycogen metabolism pathways. PTS, phosphotransferase system; PGM, phosphoglucomutase; G6P, glucose-6-phosphate; G1P, glucose-1-phosphate; ADPG, ADP-glucose.
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- Professor Graham Hatfull
- Professor Jill Banfield

Due to the evolving global medical crisis caused by the COVID-19 pandemic and its current escalation in Australia, the Australian Society for Microbiology Executive has decided to postpone the Annual Scientific Meeting that was due to take place in July 2020.