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Veterinary Microbiology

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Cover images: Photomicrograph of neural angiostrongylosis (provided by Derek Spielman), Veterinary Pathology Diagnostic Services Laboratory (provided by Mark Krockenberger), both from the Faculty of Science at the University of Sydney.

Paul Young

President of ASM

Happy New Year and welcome to the first issue of *Microbiology Australia* for 2013, the first with our new publishing partner, CSIRO Publishing. I would like to take this opportunity to express the Society's warmest thanks to Cambridge Publishing for their many years of excellent service. The transition has been a smooth one and we are genuinely excited about the new online format that has already lead to significant improvements in both accessibility and flexibility. This flexibility will allow us to more easily respond to reader suggestions – please get in touch with us if you have any suggestions for additional content or changes to the format (admin@theasm.com.au), as well as provide us with an extra avenue for more effective dissemination of information about Society and member activities. Many of you will also be pleased to hear that we have re-introduced a printed version of the journal, which will be winging its way to you each quarter. All members will receive this printed version of the journal unless you have chosen the option not to receive a hard copy when you completed your most recent yearly subscription. You can access and select this option at any time by logging in to your member profile area on the ASM home page. In consultation with all of our members, we look forward to bringing you further improvements and developments to the journal over coming issues.

Our thanks to Mary Barton, the guest editor for this edition of *MA*, who has put together an excellent series of highly informative *In Focus* and *Under the Microscope* contributions under the banner of Veterinary Microbiology. From the impact on global food security of the cross-country spread of animal diseases to the incidence of exotic bacterial pathogens in aquatic animals, the broad array of issues covered in this issue signals considerable activity in this field in Australia.

Since the last Vertical Transmission, the Society has appointed Peter Traynor as Chair of the Visiting Speakers Program – welcome on board Peter. Peter has already brought considerable organisational and coordination skills to the position and is actively working with State branches, Divisional Chairs and NSAC to build the 2013 program. If you have any suggestions for speakers who could be supported through the VSP scheme please contact Peter directly (check the ASM website) or via the national office.

The annual meeting, to be held this year in Adelaide from 7–10 July, is fast approaching – where have the first two months of the year gone! The invited international and national speakers are all in place and the tentative Program is now available for viewing at the meeting website (http://asmmeeting.theasm.org.au/). This exceptional group of speakers that the Program Committee has put together ensures that we will have a very exciting series of talks by leaders in their respective fields. While there are a plethora of smaller, specialist meetings now in play across Australia every year, the annual ASM meeting still offers the only opportunity to immerse yourself in cutting-edge science that covers the broad, cross-disciplinary field of microbiology. So set aside these three days for this important networking opportunity for all microbiologists! Early-bird registrations and abstract submissions (for oral presentation consideration) are due on the 5 April.

See you all in Adelaide in July!

From the Editorial Team

Welcome to a new era for *Microbiology Australia* with a new publisher and a return to print format in addition to a new own line version. I would like to thank *Cambridge Media* for their support in our time as editors of *Microbiology Australia*, since 2006.

In looking to the future, I congratulate the ASM Executive for making bold decisions to return to print and to improve the utility of *Microbiology Australia*, not only for ASM members but for contributors, sustaining members, advertisers and the communities we serve. When our new procedures come into full operation you can expect to receive your print issues in early March, May, September and November, while the electronic versions will be available soon after final contributions are received, which in most cases will be at least 2 weeks before the print version appears.

Our new publisher, *CSIRO Publishing*, brings a wealth of experience in publishing and you can expect to find a great deal of improvements, so I encourage you to explore both the print and on-line versions. The issues are a great resource for teaching, and some issues continue to have very high download rates to many international teaching institutions years after their first publication. I encourage teachers to refer your students to *Microbiology Australia*, and I encourage students and researchers to use the search tools to explore the resources in *Microbiology Australia.*

In terms of the operations of the journal, I refer you to the link on ASM website. In brief, the Editorial Board meets by teleconference five times each year to make recommendations about the overall direction of *Microbiology Australia*, and to decide on future issues of the journal. We are grateful for the support the Editorial Board provides with suggestions, guest editing and occasional reviewing of articles. We are also grateful to Guest Editors who are experts in their fields. They use their opportunity as Guest Editors to present the latest knowledge in their field through the selection of contributors who summarise their areas into compact articles that are readable and informative to the ASM's diverse membership. A list of future themes and their Guest Editors are shown in the box on page 7. In addition the media is informed and contributions may be used in media reports and some international websites now have direct links to some of our issues. Finally we thank the reviewers, who provide valuable comments on each contribution. They also have expertise in the articles they review, and they provide valuable comments to contributors to ensure the articles are appropriately presented.

As always, ideas and suggestions are always welcomed.

The Editorial Team: Hayley, Jo and Ian Macreadie

Veterinary microbiology

Mary Barton

Veterinary microbiology has a proud history in Australia dating back to the late 19th Century with the work of the Pasteur Institute of Sydney and McGarvie Smith on anthrax in NSW, Kendall in Victoria carrying out TB testing of cattle, Pound and his work on tick fever in Qld. In subsequent decades luminaries such as Albiston, Bull, Bennetts, Oxer, Sneddon, Turner and Carne carried out significant investigations into livestock diseases. Many others followed them. Where is veterinary microbiology heading in 2013 and beyond? In this issue Rob Rahaley describes the changing face of veterinary laboratories and notes the need for greater cooperation between government laboratories (declining in number) and private laboratories and veterinary school (both increasing in number). Veterinary microbiology has a much reduced profile in private laboratories compared with the government laboratories. Apart from providing diagnostic services microbiologists in government laboratories were responsible for much of the infectious livestock disease research carried out in Australia. CSIRO has also played a significant role. Although some work is done in veterinary schools and the surviving government laboratories it seems that research in endemic diseases is under threat. Fortunately, despite a shortage of dedicated funding, veterinary school researchers continue to contribute to investigations into companion animal infections.

The last issue of *Microbiology Australia* addressed important topics in veterinary microbiology in the context of One Health and the important role played by animals as reservoirs of zoonotic diseases. A link between that issue and this issue is seen in the paper by William Wong and Mark Schipp which addresses the problem of transboundary diseases that impact on both livestock productivity and food security for humans. This issue focusses on other aspects of veterinary microbiology and there is the full suite of disciplines – bacteriology, virology, mycology and parasitology. Unlike medical microbiology which focusses on just one species veterinary microbiology covers the full range of vertebrates other than humans and some invertebrates too and whilst some of the organisms would be familiar to many microbiologists, others are probably new. Two papers address antimicrobial resistance topics. Darren Trott draws attention to MRSA and points out that some human strains have become adapted to new hosts such as pigs and horses whereas those seen in cats and dogs have not reached this point yet. On the other hand Hanna Sidjabat and fellow researchers from the UQ Centre for Clinical Research identify the potential for multi-drug resistant Gram-negative bacteria to emerge in veterinary hospitals as well as in food producing animals. As a counter to antimicrobial resistance Kate Hodgson provides an update on use of bacteriophages to control animal diseases and as biocontrol agents. Economically significant animal diseases are covered. David Hampson provides an update on intestinal spirochaetes and on *Brachyspira hyodysenteriae* in particular which causes an important production limiting colitis in pigs and Xiaoyan Han and co-workers from the Rood laboratory report some novel work identifying a particular protease as a major virulence factor in *Dichelobacterium nodosus*, the cause of ovine footrot. Foot and Mouth disease is not present in Australia but Australian researchers such as Wilna Vosloo are working to assist our neighbours control the disease whilst at the same time developing inactivated diagnostic reagents that could be used safely in Australia. Similarly Pat Blackall provides an update on the MLST scheme for *Pasteurella multocida* which was developed for typing endemic Australian poultry strains but has now been used to type international isolates from a range of domestic and wild animals. Peter Walker's paper on bovine ephemeral fever discusses the implications of climate change on this serious arbovirus infection of cattle. Fish are not ignored and Nicky Buller reports on some of the challenges of working with fish pathogens. For those with a clinical bent Richard Malik and co-workers from the Veterinary School at the University of Sydney ask the reader to walk in the shoes of a small animal vet faced with a dog "gone in the back legs". He then discusses the role of *Angiostrongylus cantonensis* (rat lungworm) and *Neospora caninum* (a protozoan parasite) in dogs with progressive hindlimb dysfunction. For almost 50 years Des Connole provided assistance and support to veterinary microbiologists struggling with diagnosis of fungal infections as well as carrying out his own research. Justine Gibson's paper reminds us of the unique contribution Des has made to veterinary mycology. It is appropriate that Jeanette Pham's discussion of the relationship between Canadian and Australian isolates of *Yersinia enterocolitica* appears in this issue of *Microbiology Australia* as pigs carry pathogenic bioserovars of this organism in their tonsils and oral cavity.

The breadth of material presented indicates that veterinary microbiology is alive and well in Australia but under challenge. There are many animal infectious disease problems both local and international that provide challenging opportunities to future generations of veterinary microbiologists provided the jobs and funding are available.

Biography

Emeritus Professor Mary Barton AO is a veterinary microbiologist who spent two-thirds of her career in veterinary diagnostic laboratories and the last third at the University of South Australia. She has particular interests in food borne diseases, antimicrobial resistance and *Rhodococcus equi*.

Fighting transboundary animal diseases: a battle for global food security

William Wong and Mark Schipp

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Previously our team discussed the role of One Health in understanding and controlling zoonotic diseases in Australia[1]. While zoonotic diseases threaten the well-being of animals and humans and/or public confidence in food safety directly, diseases that have serious negative impact on food security through hampering various stages of our food system (from farm to fork) are also central to the role of One Health in this century. Eradication or control of these animal diseases can help ensure global food security. Interestingly many of these diseases have been around for decades or centuries and are not necessarily zoonotic by nature, e.g. foot-and-mouth disease (FMD), Peste des petits ruminants (PPR), contagious bovine pleuropneumonia, classical swine fever, Rift Valley fever, avian influenza, Newcastle disease, bluetongue and others[2]. They are commonly referred to as transboundary animal diseases (TADs) nowadays.

TADs are diseases of socio-economic, trade and/or food security significance for more than one country. They are capable of spread between countries and can reach epidemic proportions[2]. Their control and management, including eradication, requires cooperation between several countries. In the recent years, global effort to control TADs has been further challenged by increasing globalisation in animal trade and other socio-economic activities as well as ecological change. These challenges have variably and indirectly exacerbated the overall impact of TADs on livestock productivity and food security; this is an emerging area that has dominated the attention of various national and international veterinary authorities, including the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization (FAO). By 2030, global food demand is expected to increase by 50%[3]. Increased food demand places pressure on food security and means that control of TADs in production animals is even more critical, whether these animals are used for domestic or export production. It is worth noting that in many developing countries livestock species are used not only as a source of protein, but also draught animal power for cropping. Therefore, improved animal health and production contributes to greater food security as animals provide dietary protein, cash income and savings and contribute their draught power and manure toward agricultural production.

According to the OIE trade-related disease list, there are 107 diseases (or disease groups) for terrestrial animal species and 26 for aquatic animal species (including aquarium species not for human consumption)[4,5]. Among these diseases, rinderpest was officially declared in 2011 to have been eradicated globally. This represents the first animal virus (or the second virus after human smallpox) in history to have been wiped out by human effort and is one of the great achievements in modern veterinary history[6]. This global disease freedom status did not come easily; it was truly the result of continued joint effort between 198 countries and territories with susceptible animals over several decades[7]. Rinderpest historically occurred in Europe, Africa and Asia and was a TAD with high impact on food security and international trade. In 2008, FAO reported that since 1986, approximately US$610 million had been spent on animal health programs in Asia and Africa, primarily targeting rinderpest but covering other diseases and infrastructure[8]. The full spectrum of global socio-economic benefits from eradicating rinderpest is yet to be assessed but can be appreciated by the recent estimation of an at least 16-fold financial gain through reduced cattle deaths and herd productivity alone[6]. The potential annual benefit for Africa alone was estimated at US$1 billion[8]. The process and achievement of eradicating this disease clearly shows that socio-economic issues, including food security, can be a significant driving force for global actions against specific animal diseases.

Other major TADs which are still endemic in many regions worldwide and able to cause significant negative impact on food security are shown in Table 1. These diseases have long been identified by FAO and OIE as priority candidates for global eradication or control. In particular, the colossal impact of FMD on food security can be appreciated through its direct impact on the numbers of animals alone. With the current control measures in place, the worldwide numbers of major livestock affected by the disease were recently estimated (*in million per year*) at 20.59 (cattle), 0.68 (buffalo) 9.71 (sheep), 11.97 (goats) and 11.63 (pigs)[9]. During the 2001 FMD outbreak in the United Kingdom, 6.1 million livestock were slaughtered. The outbreak in South Korea in 2010–2011 also led to the destruction of more than 3 million livestock. Global production losses and the application of vaccines due to FMD can amount to US$5 billion a year[9]. Such economic impact will adversely affect other associated industries which may further worsen food security issues in some poorer countries. In Australia, FMD has been described as the single greatest disease threat to its livestock industries. The Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES) recently estimated that over a ten year period there would be severe direct economic losses (up to AU $16 billion) to the livestock and meat processing sector from an FMD

Table 1. Major transboundary animal diseases with significant negative impact on global food security.

Disease[A]	Aetiology	Major domestic animal host	Brief disease description
African swine fever	*Asfivirus* (Asfaviridae)	Pigs	Highly contagious, generalised disease with variable mortality rates
Avian influenza	*Influenza virus A* (Orthomyxoviridae)	Poultry and other bird species	Highly contagious disease characterised by respiratory, gastrointestinal and/or nervous signs in land poultry
Bluetongue	*Orbivirus* (Reoviridae)	All ruminants	Midge-borne haemorrhagic disease characterised by catarrhal inflammation of mucous membranes
Bovine brucellosis	*Brucella abortus*	Cattle, horses	Chronic disease causing abortion in cattle
Classical swine fever	*Pestivirus* (Flaviviridae)	Pigs	Highly contagious generalised disease with variable mortality rates
Contagious bovine pleuro-pneumonia	*Mycoplasma mycoides* subsp. *Mycoides* SC (bovine biotype)	Cattle, buffalo	Acute or chronic disease characterised by sero-/fibrinous pleuropneumonia
Foot and mouth disease	*Aphthovirus* (Picornaviridae)	Cattle, buffalo, pigs, sheep, goats, other cloven-hoofed species	Highly contagious vesicular disease
Newcastle disease	Serotype APMV-1 *Avulavirus* (Paramyxoviridae)	Poultry and other bird species	Highly contagious disease characterised by respiratory, gastrointestinal and/or nervous signs
Peste des petite ruminants	*Morbillivirus* (Paramyxoviridae)	Goats, sheep, cattle, pigs	Gastrointestinal and respiratory disease with high mortality rates; often subclinical infection in cattle and pigs
Rabies	*Lyssavirus* (Rhabdoviridae)	All mammals	Disease characterised by fatal encephalitis
Rift Valley fever	*Phlebovirus* (Bunyaviridae)	Sheep, goats, cattle, buffalo, camels	Mosquito-borne disease characterised by abortion and high mortalities in young animals

[A]Not a complete list.

outbreak[10]. Undoubtedly, global food security would also be compromised through a major FMD outbreak in Australia, which is one of the largest suppliers of livestock and livestock products.

Food security is often the primary driver for TAD control and eradication in developing countries while in developed countries, such as Australia, trade is a major consideration. Regardless of the type of driver, both developing and developed countries can collegially contribute to ensuring global food security through reducing or eliminating TAD related risks and threats in different ways. The Australian Government is currently developing its first ever national food plan to help ensure that the government's policy settings are right for Australia over the short-, medium- and long-term. The aim of the plan is to foster a sustainable, globally competitive, resilient food supply that supports access to nutritious and affordable food. To achieve this outcome, proposals to minimise and manage potential risks to Australia's food security and to contribute to global food security are high on its agenda[11].

Australia remains free from many of the major TADs that affect food systems around the world. Therefore, maintaining and enhancing its disease free status through strengthening Australia's preparedness for major TADs will confer significant benefits including trade and market access and global food security. Experience from rinderpest eradication has provided many valuable lessons and probably a roadmap for future global actions against other TADs. It is certain that the biological and clinical characteristics of a TAD (e.g. host range, pathogen variants, incubation period, carrier status, clinical presentations for detection, etc.) and the availability of an effective vaccine and reliable diagnostic tests are a key determinant of its suitability for global eradication or control. Undoubtedly, political willingness, supportive regulatory regimes, sustainable resources or infrastructure, strategies and coordination are vital to the ultimate success of any global TAD eradication and control program. Unfortunately, most of these institutional factors are often vulnerable to various national or international political and economic changes. In many countries, they do not have control of TADs because of poor infrastructure and investment in veterinary services governance as there are competing claims for scarce resources. In some countries, natural crises, civil unrest and wars may also have a role in further compromising their ability to control TADs.

From the veterinary perspective, there are various scientific and technical aspects that are relatively less influenced by some major institutional factors and can be enhanced to meet specific challenges for improving the chance of success in a global TAD control, eradication and/or preventive program. These aspects may include, but are not limited to, capability and capacity building through developments and innovations for field/laboratory diagnostics, vaccines/vaccination, surveillance/monitoring, communications/information systems, and training/awareness programs. In fact, some of these activities or advancements themselves may even become a driver of favourable institutional changes[2,12,13]. To further illustrate this point it is worth drawing on some of the lessons from rinderpest and smallpox eradication. Indisputably the biological and clinical characteristics of rinderpest virus did render the disease very suitable for global eradication but its success still owed much technically to the development and use of an effective vaccine and appropriate vaccination strategy as well as quality-assured laboratory testing support and various training programs[12,13]. Similarly, two particular technical elements were also highlighted as a key to the success of global smallpox eradication in humans: they are the use of an effective vaccine in a ring vaccination program and an effective surveillance program able to quickly and reliably detect emerging cases[14].

As PPR is closely related to rinderpest, it is a strong candidate for global eradication and strategic planning to this end is underway. This also shows how the flow-on benefit from one disease to another itself could become an enabler for global cooperation. From the perspective of biosecurity risk level and global food security, the TAD that is more relevant to Australia and perhaps many other parts of the world is still FMD. OIE and FAO, in response to a number of resolutions from their joint conferences on FMD held in 2009 and 2012, are currently developing the global strategy for FMD control. While the overall objective of such a strategy is to gradually reduce the incidence of FMD in endemic countries, it is hoped that this effort would eventually lead to the feasibility of eradicating the disease within the near future[9,15].

In late 2011, a report based on the review of Australia's preparedness for the threat of FMD conducted by Ken Matthews was released[16]. It contains a number of specific recommendations to enhance Australia's preparedness across the biosecurity continuum (preborder, border and post-border). The Australian Government Department of Agriculture, Fisheries and Forestry has since supported a number of projects or activities in response to these recommendations. A significant number of them involve the strengthening of national technical capacity especially for disease surveillance and response. These can be progressed without necessarily requiring substantial institutional changes. As an example, to address the need for national surge capacity for a large-scale emergency or proof-of-freedom response, a project is being developed to establish the capability for initial screening tests that involve no live virus or virus proliferation on samples in interested state/territory government laboratories for use during an FMD outbreak.

The global food security system relevant to products of animal origin is very complex and may involve areas such as availability of food, access to food and effective and safe utilisation of food[17]. There is no doubt that global food security issues will continue to shape the economic and political landscape towards livestock production, trade and biosecurity worldwide[15]. On the other hand, different economies, including Australia, will have their own contributions to

make towards ensuring global food security for public good and/or trade purposes. In this regard, while controlling or eradicating TADs plays a key role in global food security, its success largely depends on the ability to get strong commitment to regional approaches which recognise the relevant drivers in member economies. For animal health professionals, the challenge is to integrate control of TADs into broader development goals. Strengthening our scientific and technical capacity, especially through innovative technology and strategy, presents a useful way to help meet the challenge.

References

1. Coghill, A., *et al*. (2012) The role of One Health in understanding and controlling zoonotic diseases in Australia. *Microbiol. Aust.* **33**, 148–149.
2. Otte, M.J. *et al*. (2004) Livestock Policy Discussion Paper No. 9 – Transboundary Animal Diseases: Assessment of socio-economic impacts and institutional responses. February 2004, FAO Livestock Information and Policy Branch.
3. Woods, J., *et al*. (2010) Energy and the food system. *Phil. Trans. Royal Soc. – Biol. Sci.* **365**, 2991–3006. doi:10.1098/rstb.2010.0172
4. OIE (2012) OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals – Volume I and II. 7th Edition, 2012, The World Organisation for Animal Health (OIE), Paris, France.
5. The World Organisation for Animal Health (OIE) Website for OIE-listed Diseases http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2012/ (accessed 5 November 2012).
6. Anderson, J., *et al*. (2011) Rinderpest eradicated; what next? *Vet. Rec.* **169**, 10–11. doi:10.1136/vr.d4011
7. The World Organisation for Animal Health (OIE) Website for Press Release Resolution 18/2011. The world is free from rinderpest: OIE completed global free status recognition. http://www.oie.int/en/for-the-media/press-releases/detail/article/the-world-is-free-from-rinderpest-oie-completed-global-free-status-recognition/ (accessed 5 November 2012).
8. Normile, D. (2008) Driven to extinction. *Science* **319**, 1606–1609. doi:10.1126/science.319.5870.1606
9. Rushton, J. *et al*. (2012) The impact of foot and mouth disease – Supporting Document No. 1. The FAO/OIE Global Conference on Foot and Mouth Disease Control, Bangkok, Thailand, 27–29 June 2012, pp. 1–27.
10. DAFF Website for Media Release 12/44: Foot and mouth disease forum reaches agreement on measures to protect the economy. 24 August 2012, http://www.daff.gov.au/about/media-centre/dept-releases/2012/foot-and-mouth-disease-forum-reaches-agreement-on-measures-to-protect-the-economy (accessed 5 November 2012).
11. DAFF (2012) Biosecurity In: National Food Plan: Green Paper. Australian Government of Agriculture, Fisheries and Forestry, Canberra, Australia, pp. 131–133.
12. Mariner, J.C., *et al*. (2012) Rinderpest eradication: appropriate technology and social innovations. *Science* **337**, 1309–1312. doi:10.1126/science.1223805
13. Domenech, J., *et al*. (2006) Regional and international approaches on prevention and control of animal transboundary and emerging diseases. *Ann. N. Y. Acad. Sci.* **1081**, 90–107. doi:10.1196/annals.1373.010
14. The History Vaccines – Smallpox Eradication Website http://www.historyofvaccines.org/content/articles/disease-eradication (accessed 5 November 2012).
15. OIE (2011) Administrative Resolutions: The 79th General Session of the World Assembly of the OIE, 22–27 May 2011 Paris, France. *OIE Bulletin* No. 2011–4.
16. Matthews, K. (2011) A review of Australia's preparedness for the threat of foot-and-mouth disease. October 2011, Australian Government of Agriculture, Fisheries and Forestry, Canberra, Australia.
17. Bonnet, P. *et al*. (2011) Contribution of veterinary activities to global food security for food derived from terrestrial and aquatic animals. The 79th General Session of the World Assembly of the OIE, 22–27 May 2011 Paris, France.

Biographies

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Future issues of *Microbiology Australia*

May 2013: Food Safety

Guest Editor: Narelle Fegan

September 2013: Microbes and chronic disease

Guest Editor: Bill Rawlinson

November 2013: Diagnostics into the future

Guest Editors: Theo Sloots, Cheryl Bletchly, Mark Krockenburger

Some of the issues for 2014 and 2015

Microbial diseases and products that shaped the world's history

Guest Editor: Ipek Kurtböke (a joint issue with the Microbiology Society of Turkey)

AIDS

Guest Editors: Johnson Mak and Steven Kent

Coinciding with the international AIDS meeting to be held in Melbourne in July 2014.

Hospital-Acquired Infections, Susceptibility Testing and Infection Control

Guest Editors: Lyn Gilbert, Jon Iredell and John Merlino

Medical Mycology

Guest Editor: Wieland Meyer

"Gone in the back legs"

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Derek Spielman and Jan Šlapeta

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Neural angiostrongyliasis and neosporosis are the two most common infectious causes of spinal cord disease in young dogs. The former is caused by migration of *Angiostrongylus cantonensis* (rat lungworm) larvae following ingestion of mollusc intermediate hosts, while the latter is caused by the apicomplexan protozoan *Neospora caninum*, acquired transplacentally, during parturition or in the neonatal period. This article gives the reader the perspective of a veterinarian confronted with the diagnosis and treatment of these two potentially life-threatening infections, taking into account differences in epidemiology and pathogenesis, and listing diagnostic tests available and affordable for most owners. The broader implications of these infections for other species, including people and wildlife, are discussed.

Most readers of *Microbiology Australia* are scientists. In this article, we want you to leave the comfort zone of the laboratory and embrace the world of companion animal medicine. An old proverb says: to understand a man, you've got to walk a mile in his shoes, whether they fit or not. So metaphorically, we ask you to "wear" the shoes of a suburban veterinarian asked to look at a young dog who has "gone in the back legs", as many owners would put it. First we need to give you the rules of engagement, because although a profession, veterinary science is also a small business where owners expect an accurate diagnosis and a successful cost-effective solution. Good outcomes are expected even if the patient is seen at nights or at weekends, when laboratories do not offer a comprehensive service.

The dog in question is a 12-week-old Golden Retriever called "Honey" normally domiciled in Bellevue Hill (Fig. 1). She has a history of progressive hind limb dysfunction over several days. The onset was sudden, without evidence of trauma. Neurologic testing shows poor proprioceptive ability in the hind limbs, weakness that seems to be lower motor neuron (LMN) in type with poor tail carriage, urinary and faecal incontinence and marked hyperesthesia over the tail base and adjacent spine. This unusual combination of signs is most suggestive of a disease affecting the spinal cord (especially the part subserving the back end of the dog), or the corresponding nerves and/or nerve roots innervating the legs, tail and rear-quarters. Hyperaesthesia suggests meningeal inflammation. Not many diseases produce this combination of signs and common diseases such as tick paralysis (due to the toxin of *Ixodes holocyclus*) and traumatic vertebral injury do not fit the clinical picture (trust us, we are vets!). Congenital vertebral anomalies are rare in this breed and vertebral or spinal cord neoplasia is extremely rare in young dogs. So, the two most likely possibilities are neural angiostronglyosis (NA)[1–5] and neosporosis[6–11], two parasitic diseases which seem disproportionately common in eastern Australia compared to the rest of the world.

Now if a young child was presented with these signs, the investigation would likely involve a neurological consultation, perhaps an

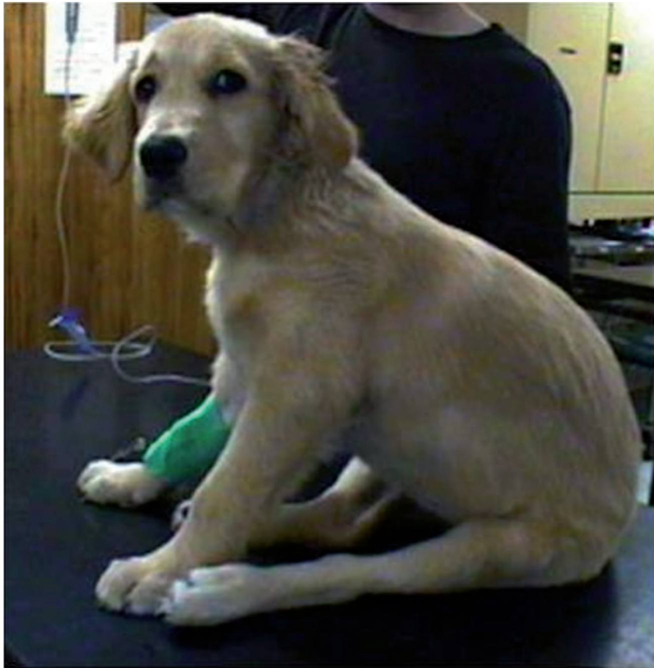

Figure 1. A 3-month-old female golden retriever presented for hind limb weakness and urinary/faecal incontinence. Eosinophilic pleocytosis and anti-*A. cantonensis* antibodies in CSF demonstrated that this dog had neural angiostronglyosis. The dog recovered completely following corticosteroid treatment and symptomatic and supportive care.

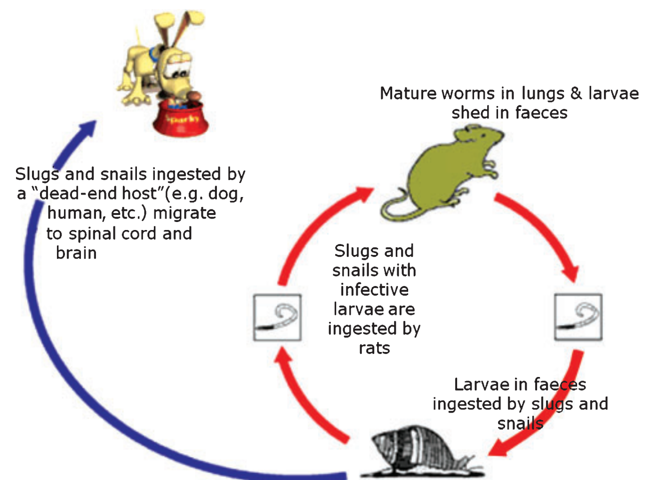

Figure 2. Life cycle of *Angiostrongylus cantonensis*[1,2,5]. The natural life cycle alternates between rats and molluscs (slugs or snails). The life cycle ends in dogs (blue line) because the ingested larvae very rarely mature into adults and no offspring are produced – it is a "dead end" for the parasite and sometimes for the host as well!

infectious diseases consultation, magnetic resonance (MR) imaging of the spinal cord and brain, electrodiagnostic tests (needle electromyography and nerve conduction studies), collection and analysis of cerebrospinal fluid (CSF), blood cultures and various serological tests[12–14]. Such a "work up" is possible for canine patients but many owners are unwilling to pay thousands of dollars to cover the cost of investigations. Unfortunately, Medicare doesn't cover dogs and most people don't take out pet health insurance. Welcome to the world of your local vet! To better understand the most likely diagnostic possibilities, to help decide which tests are most efficient, let's consider the epidemiology and pathogenesis of these infections.

Neural angiostronglyosis is a disease caused by migration of larvae of the rat lungworm *Angiostrongylus cantonensis*[1–5]. It generally occurs after a dog, typically (but not invariably) a young dog, ingests slugs or snails (Fig. 2). Anecdotal evidence from Sydney and Brisbane indicates infection of non-native rats (Norwegian and black rats) with this parasite is widespread[15,16]. Accordingly, mollusc intermediate hosts have a very high probability of harbouring infective larvae. Thus when taking a history, enquiring whether rats, snails and slugs are present in the dog's environment is pertinent, as often owners will have seen the patient ingest molluscs. The most characteristic feature of these cases is the exaggerated pain response to palpation and manipulation of the tail base, hind limbs and spine[5]. The worst cases experience excruciating pain. Signs tend to ascend, so some dogs present for nuchal rigidity referable to neck pain from parasitic meningitis, while the hind limb weakness changes neurologically from a LMN type to a mixed upper motor neuron (UMN) and lower motor neuron paresis. Later, a variety of other neurologic abnormalities, including cranial nerve palsies, may develop[5]. The prognosis with treatment is usually favourable, although some cases are left with residual neural deficits which occasionally result in euthanasia[1–3,5].

Neosporosis is a protozoan disease caused by *Neospora caninum*[6–11], an apicomplexan closely related to *Toxoplasma gondii* (the cause of toxoplasmosis)[11] and *Sarcocystis neurona* (the cause of equine protozoan myelitis, a disease of horses in North America)[17,18]. *Neospora caninum* has a very similar life cycle to *T. gondii*; however, the dog, coyote, wolf or dingo (all genetically the same species of canid) are definitive hosts for *N. caninum*, whereas the cat is the definitive host for *T. gondii*[11] and the opossum for *S. neurona*[17]. Neosporosis is mostly seen in young dogs (typically less than 6 months) infected via their dams, most likely in late pregnancy, during parturition or in the early neonatal period during lactation[6,7]; the exact mechanism is not established. After a variable latent period, pups develop signs of an ascending radiculomyelopathy, initially manifest as LMN-type hind limb weakness. If this is not diagnosed in a timely manner, the spinal cord and nerve roots subserving the hind limbs are damaged irreparably, resulting in rigidity and contracture of the pelvic limbs (Fig. 3). Spinal hyperaesthesia is usually not a conspicuous feature, although some cases have myalgia and lameness due to concurrent protozoan myositis. Pups with neosporosis can be completely cured if therapy is started early; delayed therapy results in permanent and severe neurologic deficits.

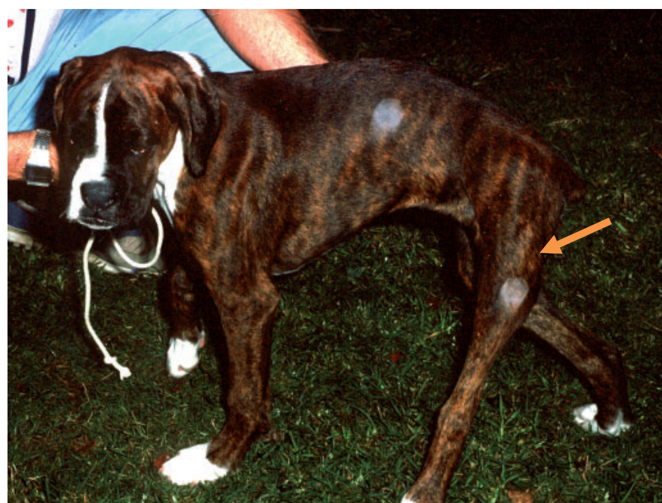

Figure 3. A Boxer pup with neosporosis. This patient was presented when there was already some contracture of the left hind limb (arrow). Treatment at this stage can arrest disease progression but the permanent loss of motor neurones means complete recovery of hind limb function is not achievable.

What clinical features are most helpful in distinguishing neural angiostrongyliasis and neosporosis? Neosporosis appears to be more common in Boxers and Labradors than other breeds, early in the disease LMN features predominate (viz. reduced muscle tone and myotatic reflexes in the hind limbs) and hyperaesthesia is not prominent. In contrast, hyperaesthesia is the conspicuous feature of NA, and the neurologic picture changes from LMN to UMN as larvae migrate "up" the spinal cord towards the brain. In both diseases, multiple littermates can be affected. In NA this is because littermates may share an environment where rats and molluscs are abundant, whereas in neosporosis siblings are affected as infection is vertically transmitted[6,7]. Both diseases are more common in large breeds[5,6]. Time of presentation can be helpful for NA, as this condition tends to be more common in autumn[5], although cases can occur in any month. Finally, geography can be an important consideration because to date NA has not been reported in Melbourne, Adelaide or Perth, although it occurs from Sydney all the way north along the coast of eastern Australia[1–5,15,16].

Two tests are most helpful in differentiating between these infections. NA is accompanied by eosinophilic meningoencephalitis, so finding eosinophilic pleocytosis in CSF is strongly suggestive of this aetiology[1–5]. The diagnosis can be confirmed by demonstrating antibodies against *A. cantonensis* in CSF using an ELISA[4,5] developed by Rogan Lee at Westmead Hospital. In dogs, CSF is generally collected from the cisterna magna under general anaesthesia. Myelography, computed tomography myelography or MR imaging can be conducted at the time of CSF collection. To date, MR studies have not been helpful in the specific diagnosis of NA in canine patients (in contrast to human cases where the presence of larval migration "trails" can be sometimes detected[9]). However, imaging studies exclude most other diagnostic possibilities. Neosporosis is normally diagnosed using an immunofluorescent antibody test (IFAT); by the time dogs are presented, they have elevated serum antibody titres against this protozoan. The IFAT is only available in laboratories in Perth (VetPath) and Launceston (Mt. Pleasant Laboratory) and assays are typically only run once or twice a week, so there is invariably some delay in obtaining a result. In neosporosis, CSF cytology shows a mixed pleocytosis, often with a prominent neutrophilic component and very rarely zoites have been recorded in CSF after cytocentrifugation[6,7].

The conundrum for the vet is that treatments of these two diseases are quite different. Neosporosis is treated with antimicrobials effective against apicomplexan protozoans, traditionally trimephoprim-sulphadiazine, pyremethamine and clindamycin[6,7]. Usually two of the three drugs are selected. The combination of sulphadiazine and pyremethamine has a theoretical advantage due to sequential attacks on the parasite's folic acid pathway. In the future, drugs such as toltrazuril and ponazuril may also prove to be effective[18], the latter having become the drug of choice for treating equine protozoan myelitis.

In contrast, NA is traditionally treated with corticosteroids to dampen the eosinophilic inflammatory response until the larvae die (because the dog is the "wrong" host)[5]. Perhaps we should be braver and actually kill the larva using an anthelmintic after starting aggressive corticosteroid therapy, choosing a drug regimen that kills larvae slowly using albendazole or fenbendazole, to avoid a sudden release of metazoan antigens. Such an approach has been used in human patients in Asia[19] and more recently in two patients at Westmead Children's Hospital[20]. Dogs with neosporosis treated with corticosteroids deteriorate dramatically. Accordingly, many veterinarians treat suspect cases (such as "Honey") with anti-protozoan drugs while awaiting serology for neosporosis, or "bite the bullet" and collect CSF to look for eosinophilic pleocytosis, if owners are agreeable. Where owners do not permit or cannot afford the full investigation, a presumptive diagnosis of NA is made after excluding neosporosis by serology or a treatment trial; corticosteroids are then administered.

It is tantalising to speculate why clinical neosporosis is apparently more prevalent in Australia than in other countries. One possibility is that it is more common to feed dogs uncooked kangaroo and beef meat in Australia (sold as "pet mince" at supermarkets) compared to other countries. Such fresh meat might contain viable cysts of both *N. caninum* (and *T. gondii*) sufficient to establish subclinical disease in the bitch, which would be transmitted subsequently to pups.

It is also fascinating that the only reports of NA in dogs have been from Australia, even though the parasite exists throughout Asia, the Pacific islands and in some southern states of the USA. This condition is behaving as an emerging infectious disease in Australia, having spread from south eastern Queensland where it was first reported by Mason in the early 1970s[1,2], reaching Sydney in 1991[3] and becoming progressively more common there[16]. Nineteen new canine cases have been diagnosed in a single referral centre in Sydney over the past 4 years (E. Thrift, A. Lam and G. Child, personal communication). It is of great interest that NA has been reported in a wide range of species other than the dog - horses[21], multiple animals in zoological collections (especially primates)[22,23], macrobats[24], possums[25] and tawny frogmouths[25,26]. From a population ecology standpoint, impact on tawny frog mouths is greater than for any other species[25,26].

From a human perspective NA is zoonotic. It occurs in patients who (accidently or purposely!) eat slugs, snails or planarians. The most celebrated Australian case was a silly chap who ate a slug on a dare at a bucks night[13]. Far more serious is the threat posed to young infants, who have the propensity to put anything into their mouths, including molluscs. An immature immune system and small spinal cord means the neurologic impact on children to be far worse than in adult patients, potentially resulting in death or permanent sequelae. For this reason, we should do much more - as scientists, doctors, veterinarians and parents – to (i) alert people to the dangers of permitting rats and molluscs to accumulate in the environment, (ii) be diligent supervising children in the backyard and (iii) educate chefs and anyone preparing salads using fresh vegetables about the need to remove slugs and snails and their mucus trails by assiduous washing. From a veterinary perspective, we need more funded research to develop anthelmintic strategies that prevent infection of dogs. Long acting moxidectin formulations given to prevent heartworm seem the most promising line for further studies[5].

Although to the best of our knowledge neosporosis has never been reported in human patients, recent evidence of infections in sheep[27], rhinos[28] and carnivorous marsupials[29] suggest that a human case will turn up sooner or later! It would be a terrible irony if the patient was a vet.

References

1. Mason, K.V. (1987) Canine neural angiostrongyliasis: the clinical and therapeutic features of 55 natural cases. *Aust. Vet. J.* **64**, 201–203. doi:10.1111/j.1751-0813.1987.tb15181.x
2. Mason, K.V., *et al*. (1976) Granulomatous encephalomyelitis of puppies due to *Angiostrongylus cantonensis. Aust. Vet. J.* **52**, 295. doi:10.1111/j.1751-0813.1976.tb00124.x
3. Collins, G.H., *et al*. (1992) Angiostronglyosis in dogs in Sydney. *Aust. Vet. J.* **69**, 170–171. doi:10.1111/j.1751-0813.1992.tb07505.x
4. Lunn, J., *et al*. (2003) Antemortem diagnosis of canine neural angiostronglyosis using ELISA. *Aust. Vet. J.* **81**, 128–131. doi:10.1111/j.1751-0813.2003.tb11071.x
5. Lunn, J., *et al*. (2012) Twenty two cases of canine neural angiostronglyosis in eastern Australia (2002–2005) and a review of the literature. *Parasites Vectors* **5**, 70. doi:10.1186/1756-3305-5-70
6. Dubey, J.P., *et al*. (2007) Epidemiology and control of neosporosis and *Neospora caninum. Clin. Microbiol. Rev.* **20**, 323–367. doi:10.1128/CMR.00031-06
7. Reichel, M.P., *et al*. (2007) Neosporosis and hammondiosis in dogs. *J. Small Anim. Pract.* **48**, 308–312. doi:10.1111/j.1748-5827.2006.00236.x
8. King, J.S., *et al*. (2010) Australian dingoes are definitive hosts of *Neospora caninum. Int. J. Parasitol.* **40**, 945–950. doi:10.1016/j.ijpara.2010.01.008
9. King, J.S., *et al*. (2012) Oocysts and high seroprevalence of *Neospora caninum* in dogs living in remote Aboriginal communities and wild dogs in Australia. *Vet. Parasitol.* **187**, 85–92. doi:10.1016/j.vetpar.2011.12.027
10. King, J.S., *et al*. (2011) Implications of wild dog ecology on the sylvatic and domestic life cycle of *Neospora caninum* in Australia. *Vet. J.* **188**, 24–33. doi:10.1016/j.tvjl.2010.03.002
11. Reid, A.J., *et al*. (2012) Comparative genomics of the apicomplexan parasites *Toxoplasma gondii* and *Neospora caninum*: *Coccidia* differing in host range and transmission strategy. *PLoS Pathog.* **8**, e1002567. doi:10.1371/journal.ppat.1002567
12. Cooke-Yarborough, C.M., *et al*. (1999) A fatal case of angiostrongylosis in an 11-month-old infant. *Med. J. Aust.* **170**, 541–543.
13. Senanayake, S.N., *et al*. (2003) First report of human angiostrongyliasis reported in Sydney. *Med. J. Aust.* **179**, 430–431.
14. Hsieh, T., *et al*. (2001) Magnetic resonance imaging findings of eosinophilic meningoencephalitis caused by angiostrongylosis. *Zhonghua Fang She Xue Za Zhi* **26**, 45–49.
15. Yong, W.K., *et al*. (1981) Localized distribution of *A. cantonensis* among wild rat populations in Brisbane, Australia. *S.E.A. J. Trop. Med. Pub. Hlth.* **12**, 608–609.
16. Stokes, V.L.D., *et al*. (2007) Occurrence of *Angiostrongylus* species (Nematoda) in populations of *Rattus rattus* and *Rattus fuscipes* in coastal forests of south-eastern Australia. *Aust. J. Zool.* **55**, 177–184. doi:10.1071/ZO06061
17. Daft, B.M., *et al*. (2002) Sensitivity and specificity of western blot testing of cerebrospinal fluid and serum for diagnosis of equine protozoal myeloencephalitis in horses with and without neurologic abnormalities. *J. Am. Vet. Med. Assoc.* **221**, 1007–1013. doi:10.2460/javma.2002.221.1007
18. Furr, M., *et al*. (2001) Efficacy of ponazuril 15% oral paste as a treatment for equine protozoal myeloencephalitis. *Vet. Ther.* **2**, 215–222.
19. Chotmongkol, V. and Sawanyawisuth, K. (2002) Clinical manifestations and outcome of patients with severe eosinophilic meningoencephalitis presumably caused by *Angiostrongylus cantonensis. S.E.A. J. Trop. Med. Pub. Health* **33**, 231–234.
20. Britton, P. *et al*. (2011) Paediatric angiostrongyliasis – two severe concurrent cases from Sydney, Australia. Proceedings of the 7th World Congress of the World Society for Pediatric Infectious Diseases, Melbourne, Australia.
21. Wright, J.D. (1991) Equine neural angiostronglyosis. *Aust. Vet. J.* **68**, 58–60. doi:10.1111/j.1751-0813.1991.tb03131.x
22. Higgins, D.P., *et al*. (1997) Neural angiostronglyosis in three captive rufous bettongs (*Aepyprymnus rufescens*). *Aust. Vet. J.* **75**, 564–566. doi:10.1111/j.1751-0813.1997.tb14194.x
23. Carlisle, M.S., *et al*. (1998) Cerebrospinal angiostronglyosis in five captive tamarins (*Sanguinus* spp). *Aust. Vet. J.* **76**, 167–170. doi:10.1111/j.1751-0813.1998.tb10121.x
24. Barrett, J.L., *et al*. (2002) Neuro-angiostrongylosis in wild Black and Grey-headed flying foxes (*Pteropus* spp). *Aust. Vet. J.* **80**, 554–558. doi:10.1111/j.1751-0813.2002.tb11039.x
25. Ma, G., *et al*. (2013) Tawny frogmouths and brushtail possums as sentinels for *Angiostrongylus cantonensis*, the rat lungworm. *Vet. Parasitol.* in press. doi:10.1016/j.vetpar.2012.11.009
26. Gelis, S., *et al*. (2011) Neuroangiostrongyliasis and other parasites in tawny frogmouths (*Podargus strigoides*) in south-eastern Queensland. *Aust. Vet. J.* **89**, 47–50. doi:10.1111/j.1751-0813.2010.00660.x

27. Bishop, S., *et al*. (2010) The first report of ovine cerebral neosporosis and evaluation of *Neospora caninum* prevalence in sheep in New South Wales. *Vet. Parasitol.* **170**, 137–142. doi:10.1016/j.vetpar.2010.01.030
28. Sangster, C., *et al*. (2010) Neosporosis in an aborted Southern White Rhinoceros (*Ceratotherium simum simum*) foetus. *J. Wildlife Zoo Med.* **41**, 725–728. doi:10.1638/2009-0250.1
29. King, J.S., *et al*. (2011) Extensive production of *Neospora caninum* tissue cysts in a carnivorous marsupial succumbing to experimental neosporosis. *Vet. Res.* **42**, 75. doi:10.1186/1297-9716-42-75

Biographies

Richard Malik is a consultant in small animal medicine that has a special interest in infectious diseases of companion animals. He is particularly interested in viral diseases of cats, fungal diseases especially those caused by *Cryptococcus* species, mycobacteria, saprophytic pathogens such as *Burholderia*, *Prototheca* and *Pythium* and most recently parasitic diseases because of the influence of Jan Slapeta! Richard works for the Centre for Veterinary Education where he facilitates feline distance education programs, and develops life-long learning strategies for vets in practice.

Derek Spielman is a Lecturer in Veterinary Pathology at the University of Sydney. He has worked as a companion animal clinician, zoo and wildlife veterinarian and has a PhD in conservation genetics. His special interests are in wildlife pathology and the ecology and epidemiology of wildlife diseases. He is also interested in the pharmacokinetics of antibiotics in Australian marsupials. He is especially interested in the effects of neural angiostronglyosis on tawny frogmouths and possums.

Jan Šlapeta joined the Parasitology team in the Faculty of Veterinary science at the University of Sydney in 2007. He has a broad understanding of the biology of parasites of both medical and veterinary importance, as well as the diseases caused by them. He has experience in several research laboratories, including the NIH in the USA, the CNRS in France and the University of Technology in Sydney. Jan specialises in the molecular identification and the evolution of protozoan parasites. His diagnostic techniques and biodiversity studies have received worldwide interest. He has a particular interest in applications of molecular biology towards elucidating the unique properties of parasites of medical and veterinary importance.

The changing roles of veterinary laboratories in Australia

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Over the past 30 years there has been a major restructure of government veterinary laboratory services in Australia coinciding with, but not directly related to, the proliferation of private veterinary laboratories. State and territory government services have been increasingly centralised with greater focus on surveillance for exotic and emerging animal diseases and a shift away from animal health research. Private pathology services have flourished as veterinary practitioners increasingly value laboratory support for their clinical assessments and animal owners are prepared to spend more for the care of their pets. Future challenges and opportunities exist for governments to maximise return on investment in laboratories through minimising duplication of services, leveraging the academic and infrastructure resources of university veterinary schools and better utilising the efficiencies of the private sector.

Government laboratories

The need for laboratory support for government animal health programs was recognised early in Australia's colonial history. Laboratory-based veterinary diagnosis was recorded as early as 1890[1] and the first dedicated veterinary diagnostic and research facility, the Queensland Stock Institute, was established 1893[2]. Other government funded, veterinary-specific laboratories began to appear in the early 1900s and by 1925 all states had at least a basic veterinary laboratory service with one or more pathologists[3].

The laboratories played an important role in disease control and research and greatly assisted the development of Australia's

Figure 1. Mobile laboratory used during pleuropneumonia eradication campaign in the Northern Territory.

livestock industries as producers adapted to the many animal health challenges of managing livestock in an often harsh environment. Anthrax, clostridial diseases, tick fever, brucellosis, tuberculosis and internal parasites were just a few of these challenges. Laboratory diagnostic support was an essential part of the fight against bovine pleuropneumonia which had ravaged Australian cattle herds until its eventual eradication in 1968[4].

The commencement of the bovine brucellosis and tuberculosis eradication campaigns (BTEC) in 1970[5] heralded a period of unprecedented expansion of government laboratories. Brucellosis eradication required testing of millions of blood samples and regional laboratories complemented the work done in central laboratories. In both the pleuropneumonia and BTEC programs, caravans converted to mobile laboratories were also used to handle the large number of samples in remote areas (Fig. 1).

By the mid-1980s Australia had 23 "bricks and mortar" veterinary laboratories scattered throughout the country (Table 1). In addition to the BTEC work, these laboratories also performed general diagnostic pathology and were an important part of the overall national animal disease surveillance system.

Happy days

The early 1980s are often viewed by aging veterinary diagnosticians as the halcyon days of veterinary laboratories in Australia. In addition to the massive activity generated by the BTEC governments generally accepted a role in supporting livestock production through extension activities, control of endemic diseases and a significant level of research. Access to diagnostic services was free and laboratories were well staffed.

Surveillance for diseases was also a key priority. Information on what diseases are, or are not, present in Australia is essential to underpin our interstate and overseas market access and for protection of public health. Australia is free from many of the serious infectious diseases affecting livestock in other parts of the world and, in particular, the absence of foot and mouth disease (FMD) is critical to our overseas trade access. FMD surveillance has always been a major focus for government laboratories.

A critical step in early detection of exotic and emerging diseases is farmer reporting of abnormalities in their stock. The provision of free laboratory services was a major incentive for reporting and the regional location of many of the laboratories facilitated submission of whole animals for post-mortem examination. Most regional laboratories had a healthy caseload and this provided an excellent training ground for laboratory diagnosticians.

Despite this extensive national laboratory network, Australia still lacked the capability to actually diagnose many exotic diseases. Without a high security laboratory, live viruses required for many diagnostic assays could not be held in the country and suspect samples had to be sent overseas to laboratories such as Pirbright in the UK.

Following considerable lobbying from industry and the veterinary profession, the Australian Animal Health Laboratory (AAHL), a world class, high security biocontainment laboratory was commissioned

Table 1. Australia's network of veterinary laboratories in 1990.

Jurisdiction	Location of laboratories
Victoria	Parkville, Attwood, Hamilton, Benalla, Bairnsdale, Bendigo
NSW	Menangle, Wagga Wagga, Wollongbar, Armidale, Orange
WA	South Perth, Albany, Bunbury
NT	Darwin, Alice Springs
SA	Adelaide, Struan
Queensland	Brisbane, Rockhampton, Townsville, Toowoomba
Tasmania	Mt Pleasant

and opened in 1985 at a cost of more than $185 million[6]. AAHL continues to play a pivotal role in Australia's defence against the incursion of exotic animal disease and is at the forefront of research into both exotic diseases and emerging animal diseases such as Hendra virus and bat lyssavirus. In recent years, AAHL has adopted a strong "One Health" focus to leverage the value of cooperation between medical and veterinary investigators.

The emergence of AAHL was not without controversy however, and an acrimonious, and ultimately successful, campaign was mounted to prohibit the laboratory housing live FMD virus for fear of its escape from the facility. This ban remains in place today, severely impeding AAHL in achieving its mission. The development of molecular diagnostics and antigen capture techniques now enables the diagnosis of clinical FMD at AAHL but research into this important disease remains confined to off-shore laboratories at considerably greater cost.

The wheels start to wobble...and fall off

Australia was declared provisionally free of bovine brucellosis in 1986 and provisionally free of bovine tuberculosis in 1992[5]. The BTEC work and associated industry funding that underpinned many of the regional laboratories evaporated and, in the late 1980s, governments were faced with an increasing financial burden to maintain the laboratories. New terms like "cost recovery", "beneficiary pays" and "rationalisation" started to creep into conversations.

South Australia was the first state to move on the cost of their laboratory network closing the Struan laboratory in 1991 and, in the same year, the Arid Zone Research Laboratory in Alice Springs closed. In 1994, the Victorian government negotiated with a private consortium to outsource the management of all of their 4 regional laboratories. The Hamilton, Benalla, Bendigo and Bairnsdale laboratory buildings were leased to the consortium but the Department of Agriculture retained control of the Victorian Institute of Animal Science at Attwood. This was fortunate because the outsourcing was a disaster. In 1996, the Department initiated another tender process for provision of diagnostics services, with the successful tenderer operating from their own central pathology laboratory. Consequently, the consortium ceased operations and dismissed all staff at the regional laboratories[7].

NSW also reduced their number of laboratories closing the Wagga and Armidale laboratories in 1996. Wollongbar and Orange closed in 2009. In 1997, the South Australian government outsourced its sole remaining state laboratory to a private company. Bunbury in WA closed in 1999.

Closures continue, with the Queensland government recently announcing the closure of their Toowoomba and Townsville laboratories and centralisation of all testing in Brisbane. If these closures proceed, only 7 of the 23 laboratories will remain.

A new paradigm

Despite the drastic reduction in regional laboratories, the value of veterinary diagnostics has not been lost on government and investment in central laboratories continues. The Berrimah laboratory in Darwin was rebuilt in 1998 and a new laboratory was constructed at Coopers Plains in Brisbane in 2010. In NSW, a $57 million upgrade of the Elizabeth McArthur Institute of Agriculture was opened in 2012. A more ambitious investment is the recently completed Centre for Agribioscience in Victoria (Fig. 2). A joint venture between the Victorian government and Latrobe University, this $288 million

Figure 2. Victoria's new Centre for Agribioscience.

complex now houses Victoria's veterinary diagnostic laboratory (formerly at Attwood) alongside other bioscience activities[8]. The WA government has proposed to rebuild the South Perth veterinary laboratory in 2014–16[9].

Another positive in the reduction of the number of laboratories is that all government laboratories are now NATA-accredited, providing an external assessment of quality assurance. Maintenance of quality assurance systems is very resource demanding and it's doubtful that many of the regional government laboratories could have undertaken this within existing budgets.

Concurrent with the centralisation of laboratory services there has been a significant shift in government policy regarding the application of public funds to support livestock industries. State government funding for endemic disease control programs has all but ceased and there is little state government money allocated to animal health research. Instead, laboratories are more focused on surveillance for emerging and exotic diseases with other work usually done on a user-pays basis. For the most part, with the exception of AAHL, research has either disappeared or gravitated away from government laboratories to universities. Not only has this reduced the overall amount of animal health research being undertaken in Australia, it has also fractured the important link that existed between contemporary diagnostic activity and applied research into diseases of production animals.

Follow the LEADDR

A recent initiative for state laboratories is the Laboratory Emergency Animal Disease Diagnosis and Response program (LEADDR). The 2007/8 incursion of equine influenza virus into Australia and subsequent successful eradication campaign demonstrated the value of a distributed capacity for diagnosis of an exotic animal disease, particularly during the proof of freedom phase which may involve testing many thousands of animals. The LEADDR program involves transfer of non-agent replication technology such as PCR and ELISA from AAHL to the state laboratories under an umbrella of strict quality assurance.

Private laboratories

Private veterinary laboratories have a much more recent history in Australia. Medical laboratories have dabbled in veterinary diagnostics in the past, particularly in regional centres, but this has been more of a service to the local veterinarians rather than a bona fide business venture.

The first private veterinary laboratory owned and operated by a qualified veterinary pathologist opened in Melbourne in 1979. A second, unrelated business commenced in Brisbane in 1985. These laboratories focused on the pet animal and racehorse markets and both of the pathologists had strong personal following. Business growth was initially slow but increased as veterinarians' appreciation and reliance on laboratory support for their diagnostic work-up increased and the public became more willing to spend on their pets. Similar enterprises were soon established in Sydney, Adelaide and Perth.

Today there are about 20 private laboratories servicing the Australian veterinary industry, primarily in capital cities but a few are in regional centres. They may offer a full range of diagnostic services or specialise in disciplines like parasitology or trace mineral analyses. In some cases they are associated with medical laboratories, leveraging the existing infrastructure. While sharing couriers, buildings and some analytical equipment is a logical and very successful business model, animal pathology and microbiology are often very different from their human counterparts and dedicated staff and equipment are often required.

The primary objective of a private laboratory is generating profit. As a result, both the caseload and the work environment are very different from their government counterparts. In most private laboratories, samples from companion animals and the racing industry predominate with the bulk of revenue generated from haematology, biochemistry, histopathology and cytology. Most operate or access courier services and there is generally a greater urgency to get results out to appease very demanding veterinary clients. High volume throughput is the key to success and automation is a priority to minimise labour costs. Post-mortem examinations are expensive if they are charged at true cost-recovery and are therefore uncommon.

There have been a few instances where private sector laboratories have encroached on the traditional business of government laboratories. The private business environment is generally more flexible and can allow a more responsive approach to labour requirements through better utilisation of casual staff. This, combined with better adoption of automation, makes them a financially attractive option for high volume testing such as serology and they have competed successfully for export testing of farm animals and testing for disease control programs such as ovine brucellosis, enzootic bovine leucosis and Johne's disease.

The profit motive does not mean that private sector laboratories compromise on quality or service. In fact it's quite the opposite. The business is highly competitive and provision of a high quality service is essential for survival. Most large private veterinary laboratories are NATA-accredited and the private sector employs more specialist veterinary pathologists than the public sector.

Unlike their medical colleagues, many veterinary practitioners also have quite sophisticated in-clinic laboratories. Bench-top analysers allow on-site haematology, biochemistry and endocrinology. These units have become increasingly sophisticated and accurate over the past 15 years and they have been widely adopted as part of routine clinical work-ups. The uptake of these analysers by veterinarians was once viewed as a threat by private laboratory operators; however, they have also led to an increased awareness and appreciation of clinical pathology by both veterinarians and animal owners so a peaceful coexistence has developed. One major provider of referral veterinary laboratory services in Australia is also the major vendor of in-clinic analysers and their business model is to view the options as complementary rather than competitive.

Private versus public – cooperation or competition?

The advent of private veterinary laboratories was viewed with both cynicism and concern by many government laboratory personnel. The profit motive was seen by some as compromising science. At the time, a significant number of government laboratories also provided some level of service for small animal veterinary practitioners to bolster their own budgets and they did not appreciate this new competition. Conversely, the private sector viewed the government laboratories as unfairly competing in the market place with marginal costing policies resulting in less than commercial fee structures. They argued that the government had no role in servicing the pet and pleasure animal markets.

Fortunately, today the public and private sector have a much more comfortable relationship as they have realised that their roles are different. Government laboratories have essentially exited the companion animal market but there are still occasional grumblings about private laboratories "cherry picking" profitable farm animal services. Nonetheless, each sector appears to have found its niche and now realise that they can coexist. Competition for qualified staff from the private sector has had some positive impact on salaries for government laboratory workers.

In a few cases, private laboratories have replaced government operated services. Victoria continues to outsource some testing (primarily serology) to a private laboratory but retains a central government laboratory for core diagnostic work. They have persisted with this model since 1996. In South Australia, the private sector has provided all laboratory services to government for the past 15 years. This model is not without its challenges as the work environment of a commercial fee-for-service laboratory does not afford pathologists and other laboratory diagnosticians much reflection time. When a diagnostic sample is sent to a laboratory, government's requirement is that the case is examined as a whole and the implications of the findings for the state or territory's animal health, trade, public health and environment are all considered. In a commercial environment, the diagnostician has little time for such thinking before moving on to the next case. One solution is to build this "thinking time" into the negotiated fee structure but this tends to negate the assumed cost-savings of privatisation.

Universities

There are now 7 veterinary schools in Australia and all must have access to laboratories to support their clinics and for teaching. All have highly credentialed laboratory diagnosticians and all struggle to maintain an appropriate caseload of farm animal species.

To leverage both expertise and case material, there have been several attempts to co-locate government laboratories with university veterinary schools. This is a common model for service delivery in the Unites States where state-funded diagnosticians operate side-by-side with university teaching and research staff. To date, all proposals in Australia have been abandoned before their implementation. Perhaps this reflects the difficulty in aligning the teaching and research functions of a university with the surveillance and response functions of government but this can be overcome by resourcing appropriately to the diagnostic caseload. It would be prudent for further attempts to be made to make it work here. Universities benefit from the added caseload (and therefore teaching material) that government laboratories attract and government benefits from both the expanded expertise found in an academic institution and the recognition of "teaching value" that universities apply in setting cost-recovery models. Both benefit from the efficiency of shared facilities and a more robust scientific environment. The joint venture between the Victorian government and Latrobe University may provide further insight into how this might work in the Australia. Although Latrobe does not have a veterinary school they do offer an undergraduate degree in animal and veterinary bioscience.

Challenges for the future

The reduction in government veterinary laboratories is an irreversible trend and further rationalisation of services is likely as governments continue to scrutinise their expenditure of public funds. However, this process needs to be better coordinated. While jurisdictions have taken steps to maximise the efficiency of services within their boundaries, there is still significant duplication of resources and services across jurisdictions. If all state and territory laboratories were under a single controlling agency, the optimal national network of laboratories to support government

surveillance and disease control programs would look quite different to what we have today.

Rationalisation comes at some cost however. Training opportunities for veterinary laboratory diagnosticians have been reduced and there are concerns about the future availability of a qualified workforce. The closure of regional laboratories and the fee structure of the private laboratories have resulted in a dramatic reduction the number of post-mortem examinations undertaken by trained pathologists in Australia. Most are now done in the field by veterinary clinicians who submit tissue samples to the laboratories. No doubt disease diagnoses are delayed or missed because of this.

There is a need to improve information exchange between all laboratory sectors. Governments need diagnostic information to build an overall disease surveillance profile for the country. This information is fed into a national animal health surveillance database and used to underpin trade negotiations. For the most part, university and private laboratory data is lost to this system. Conversely, private and university laboratory diagnosticians would benefit from more information about what is being seen by their government colleagues.

Another key challenge for both government and private laboratories is how to better integrate their services. Private sector (and university) laboratories are now represented on the Subcommittee on Animal Health Laboratory Standards reporting to Animal Health Committee but there needs to be more consideration of how governments might tap into the potential of the private sector, for example in provision of high volume testing during an emergency animal disease response. Developing competitive tender contracts for provision of services before an emergency would most likely be very cost-effective in a national animal disease emergency.

The National Animal Health Laboratory Strategy (NAHLS)

To address many of these issues, Animal Health Australia (AHA) was requested by governments to convene a strategic advisory group in 2006 to develop a national strategy to rationalise laboratory service delivery in Australia. The mission of the NAHLS was the development and delivery of a national animal health laboratory service capability for the effective control of animal diseases of major importance to Australia. The strategy was to look at strengthening diagnostic capability through adequate resourcing, staff training, enhanced diagnostic technology and improved information technology. The advisory group included government, university and private sector representation.

Unfortunately, despite considerable effort by AHA, little progress has been made. Some laboratory personnel viewed the strategy as a threat and it was difficult to drive strategic change in a system without control of the sources of existing funding or any significant additional funds. The strategy did not engage senior decision makers and many state governments continued to make major investments in laboratories without national consultation or coordination.

Perhaps a different approach is needed; instead of paradigms like rationalisation and efficiency the answer may be to work towards expanded centres of excellence where the focus could be technologies, species or diseases. Such a system need not preclude wider service delivery to fulfil surveillance needs but would focus significant government expenditure on key national priorities and minimise duplication of effort. A shared vision between governments and universities and better information flow to reignite the synergy between diagnostic pathology and research is probably more important than shared infrastructure. Both need to recognise the strength of the private sector in the cost-effective provision of high throughput services.

Government, private and university laboratories all have different drivers, skills and strengths but if a truly national animal health laboratory strategy is to work, what better than centres of excellent embracing private, pubic and university laboratories, each doing what they do best?

Acknowledgements

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The views expressed in this article are those of the author.

References

1. Mylrea, P.J. (1989) The laboratory diagnosis of diseases in livestock in New South Wales: 1890 to 1947. *Aust. Vet. J.* **66**, 114–117. doi:10.1111/j.1751-0813.1989.tb09762.x
2. Angus, B.M. *et al*. (2007) Parasitology and the Queensland Museum, with biographical notes on collectors. *Memoirs of the Queensland Museum* (Brisbane: Queensland Museum) **53**, ISSN 0079-8835
3. Vallis, R. (2011) A Veterinary Awakening: The History of Government Veterinarians in Australia. Dept. of Agriculture, Fisheries and Forestry Canberra.
4. Newton, L.G. and Norris, R. (2000) Clearing a continent: The eradication of bovine pleuropneumonia from Australia CSIRO Publishing ISBN 0 643 06562 8.
5. Lehane, R. (1996) Beating the odds in a big country: The eradication of bovine brucellosis and tuberculosis in Australia. CSIRO Publishing ISBN 0 643 05814 1.
6. www.csiro.au/en/Organisation-Structure/National-Facilities/Australian-Animal-Health-Laboratory/AAHL-Profile/History-highlights.aspx

7. Anon. (2003) Inquiry into veterinary pathology services report. Parliament of Victoria Environment and Natural Resources Committee. p15
8. www.agribio.com.au
9. www.agric.wa.gov.au/objtwr/imported_assets/aboutus/corp/renewal_faqs_external_final.pdf

Biography

Dr Rob Rahaley is South Australia's Chief Veterinary Officer. He is also a specialist veterinary pathologist and worked for 15 years in the Victorian veterinary laboratory system before becoming a partner in Veterinary Pathology Services, Australia's largest private veterinary laboratory, and managing their Adelaide laboratory for 12 years. During this time his company was awarded the inaugural contract to provide veterinary laboratory services to the South Australian Department of Primary Industries. The company was sold to IDEXX Laboratories in 2000 and in 2002 he moved to the UK as manager of the IDEXX laboratory at Wetherby. He returned to Australia in 2005 as the Australian and New Zealand Country Manager for IDEXX. Dr Rahaley was appointed the South Australian Chief Veterinary Officer in 2007. He is a member of the Australian Animal Health Laboratory Strategic Policy Group and is an Affiliate Professor within the School of Animal and Veterinary Science at Adelaide University.

Foot-and-mouth disease: a persistent threat

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Foot-and-mouth disease (FMD) is a viral infection of cloven-hoofed animals. It is considered one of the most infectious viral diseases known and is feared for its ability to spread rapidly and cause widespread outbreaks in domestic livestock under intensive farming conditions. Remarkably, it does not cause high mortality, but morbidity can reach 100%. The disease has been eradicated from large parts of the world, and countries that are free of FMD take extreme precautions to prevent its reintroduction. For this reason FMD has been called an economic disease due to resultant trade restrictions and subsequent losses in income that have been estimated to reach between $7.1–16 billion for Australia depending on the size and duration of the outbreak[1].

Foot-and-mouth disease virus belongs to the genus *Aphthovirus* in the family *Picornaviridae* and exists as seven distinct serotypes (O, A, C, Asia-1 and South African Territories (SAT) 1, 2 and 3) with the latter 3 belonging to a different lineage. There is little to no cross-protection between virus isolates belonging to the different serotypes, complicating control of the disease when using vaccines. It is a single-stranded RNA virus with a small genome (~8.5 kb) that lacks proofreading ability[2] and each serotype therefore exists as numerous genetic and antigenic variants that have been classified into topotypes, i.e. geographically linked viruses with limited genetic variation[3]. The geographic distribution of the serotypes varies and different regions have their particular pools of viruses for which specific vaccine strains are needed. So far, seven pools of viruses have been identified to assist with control plans[4] (Fig. 1).

The disease has a very wide host range and most cloven-hoofed species are susceptible, although at varying levels[5]. However, their importance in the maintenance and spread of the infection varies depending on various factors such as the species of animal involved, the virus isolate, the infectious dose and the immune status of the animals. For example, there are FMD virus isolates that are highly infectious to pigs, but not cattle[6], while sheep and goats rarely show overt clinical signs[7]. Impala (*Aepyceros melampus*) that are found in sub-Saharan Africa are sometimes referred to as indicator species due to their high susceptibility to infection. During infection, that could also be sub-clinical, they can transmit the disease to other susceptible species, but factors such as animal density and contact rates determine that impala do not play an equally important role in the epidemiology of the disease in all regions where the species occur[8]. The African buffalo (*Syncerus caffer*), that is limited to sub-Saharan Africa, mostly suffer sub-clinical infection. It is the only species that has been shown to maintain the three SAT serotypes of FMD for long periods of time probably due to co-evolution of host and virus. The virus is present in cells obtained from oro-pharyngeal scrapings more than 28 days after the clinical phase of the disease has ended[9]. Although it is not clear how buffalo can transmit the disease, there is sufficient evidence that they can act as a source of infection for other domestic and wildlife species[10,11].

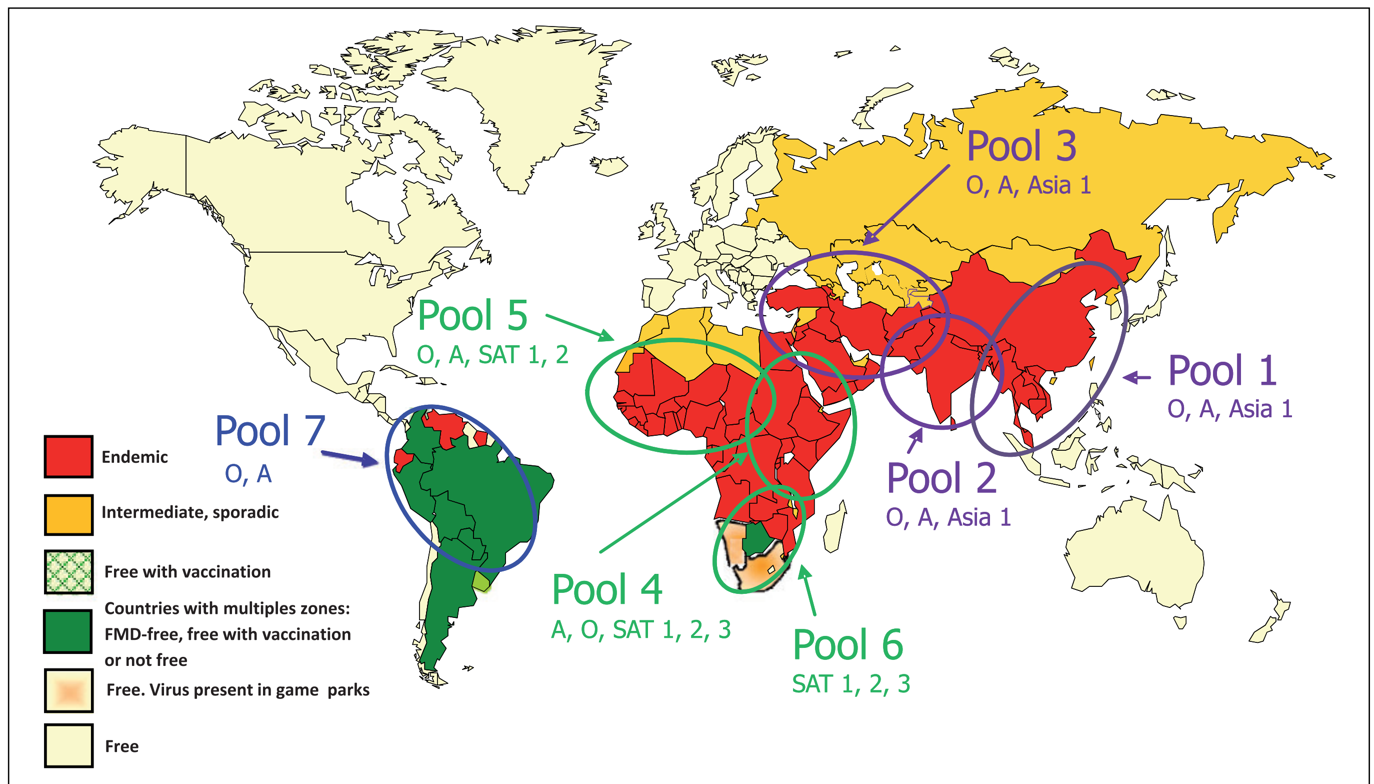

Figure 1. Map indicating the prevalence of foot-and-mouth disease internationally and the approximate distribution of the 7 virus pools that have been identified to assist with control of the disease (kindly provided by J. Hammond, FMD World Reference Laboratory, Pirbright Institute, United Kingdom).

The clinical signs appear in epithelia subject to friction, such as the tongue, the coronary band and the interdigital space of the hoof as well as the teats of lactating animals (Fig. 2). In severe cases, the hoofs can slough. Mortalities occur typically only in young animals as a result of myocarditis, referred to as tiger heart disease. Most animals clear the virus from all excretions within 14 days as the levels of neutralizing antibodies increase, but ruminants can become persistently infected with FMD virus for variable periods of time where virus can be found, sometimes intermittently, in the germinal centres within the dorsal soft palate, pharyngeal tonsil, palatine tonsil, lateral retropharyngeal lymph node and mandibular lymph node[12]. However, persistently infected domestic animals have not been shown to transmit the infection experimentally while only anecdotal evidence exists of them doing so under natural conditions, and their role in the epidemiology of the disease is still a matter of contention[13]. Although most animals survive the infection and lesions heal in a relatively short period of time, loss of condition and overall productivity including decreased milk production can result. The greatest impact is seen in intensive farming systems such as feedlots, dairies and piggeries.

Pigs are considered to be amplifier hosts as they excrete up to a 1000 times more virus into the environment than infected cattle[14]. Cattle are more susceptible to airborne infection, most likely due to their large tidal volume. Virus can be present in secretions and excretions up to 4 days before clinical signs are evident (reviewed in Thomson and Bastos[15]) making products such as milk particularly hazardous as it could contain virus and be distributed widely before any movement control or quarantine measures are in place.

Hand, foot and mouth disease that affects predominantly children is caused mostly by *coxsackie A virus* and *enterovirus* 71, both from the *Picornaviridae* family[16]. This infection is often confused with FMD. The FMD virus is not a zoonotic agent, therefore products that contain FMD virus are safe for human consumption, although it is not good practice to allow such products into the human or animal food chain. However, these products could be infectious when fed to susceptible animals. Several mitigation steps are available to render products free of infectious virus for trade purposes, such as ultra high heat treatment for milk[17] and allowing the pH of meat, especially beef, to decrease below pH6.0 whilst also removing high risk material such as bone and lymph nodes[18]. These measures are being advocated to allow commodity-based trade from regions where it is difficult to control FMD and to gain better access to export markets[19].

FMD is endemic to large parts of South East Asia (SEA) where serotypes O, A and Asia-1 are prevalent. The southern parts of the region such as Indonesia, the Philippines, Singapore and East Malaysia are free of the disease. Under the auspices of the World

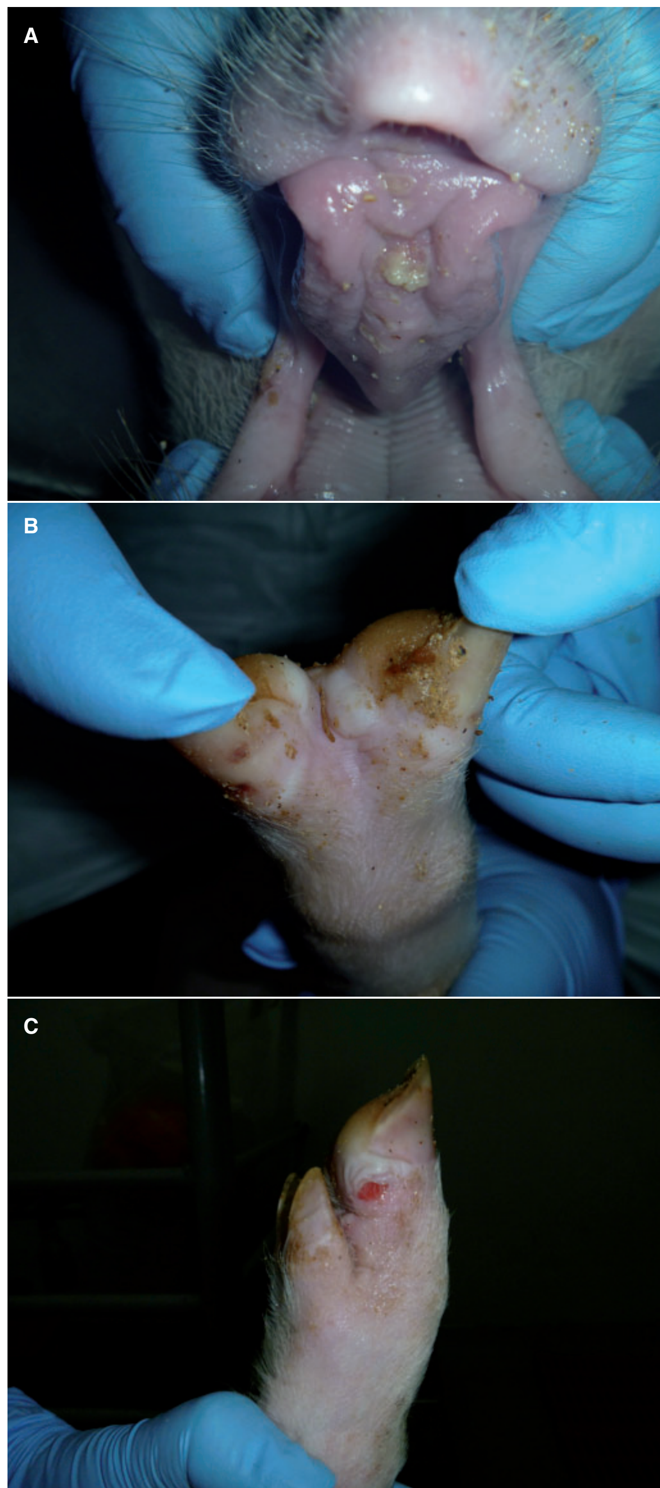

Figure 2. Lesions in pigs experimentally infected with FMD virus. (A) Vesicles on the dorsal side of the tongue of an infected pig. (B) Lesion in the interdigitary space. (C) Ruptured vesicle on the coronary band.

Organisation for Animal Health (OIE) a regional plan is in place to improve control of FMD progressively and so assist countries to improve productivity and access export markets. Regional control will in turn lower the risk of accidental introduction for Australia. Due to the large number of people moving between the two regions and the risk of illegally imported foodstuffs, SEA is seen as the biggest risk of accidental introduction of FMD into Australia.

The current vaccines consist of concentrated and inactivated virus formulated with adjuvants; the vaccines need to be administered annually or more frequently, depending on the adjuvant, to ensure protective herd immunity in vaccinated livestock (reviewed in Thomson and Bastos[15]). Countries such as Australia that are free of FMD, keep emergency antigen banks that could be used in the case of an outbreak. These vaccines will be of high potency to induce a rapid immune response and will most likely be administered only once to protect animals against disease and suppress virus circulation to augment the other control measures such as stamping out, quarantine and movement control. Australia's FMD control plan (AUSVETPLAN)[20] is available for scrutiny online and has been used as example by several other countries to develop their own control plans.

It is important to determine whether vaccines will be efficacious against viruses circulating in the field. Currently there are a limited number of vaccine strains available internationally due to the difficulty of adapting FMD virus isolates as effective vaccine strains. Added to that, viruses are continuously mutating and recombining, giving rise to new variants that can escape vaccine immunity (see below). It is therefore important that all countries and regions be vigilant to monitor for potential new variants and ensure vaccines are efficacious. In recent years there have been a number of such incidents, for example new type A variants occurred in Argentina in 2000 and the spread of A-Iran-2005 throughout the Middle East led to the need to develop new vaccine strains[21,22].

The gold standard for testing vaccine efficacy remains performing potency tests in animals. Although neutralizing antibodies are important predictors of protection for homologous challenge, the titre of antibodies are not as reliant when predicting protection against heterologous challenge. New methods such as determining the avidity and sub-types of antibodies and cell mediated responses are being investigated to provide more accurate correlates for protection[23,24].

Diagnostic tests are available to detect virus antigen, genomic material and antibodies to FMD virus[25]. However, due to the significant amount of variation between and within serotypes, pan-serotypic diagnostic assays are essential for index case diagnosis and the polymerase chain reaction-based assays that detect conserved regions of the genome are especially valuable in this regard. In addition, these assays are amendable for high-throughput diagnostics, where many samples have to be tested during an outbreak[26]. To date, it has not been possible to design serological assays that can detect all serotypes and the variants within serotypes and the focus will be on pool specific assays (Fig. 1). The serological

assays that are used to distinguish between vaccinated and infected animals are based on the differentiation of antibodies to the structural- (SP) and non-structural proteins (NSP) of the virus; the latter are conserved between the serotypes. NSP tests are therefore useful for detection of infection both in vaccinated and unvaccinated animals. However, the sensitivity and specificity of these tests depend on the immune status of the animals; tests being less sensitive when animals have been vaccinated prior to becoming infected[27].

Australia has not had an outbreak of FMD since 1872 and has significant trade advantages due to its freedom, not only from FMD, but also from other infectious diseases. This benefit needs to be protected at all costs, hence a need for both post- and pre-border mitigation of risks. Part of this objective is to ensure the country is prepared for a disease emergency; therefore, the Australian Animal Health Laboratory is executing a project under contract with Animal Health Australia and industry funding via Meat and Livestock Australia to test the efficacy of available vaccine strains in the antigen bank against viruses isolated recently in SEA. All this work has to be performed offshore in facilities that are allowed to work with live FMD virus through international research collaborations. The project also focuses on studying the pathology of the viruses in Australian breeds of cattle, sheep and pigs, validating diagnostic tests and studying the molecular epidemiology of FMD in the SEA region. This project will provide useful information vital for laboratory based surveillance and control actions.

References

1. http://www.daff.gov.au/animal-plant-health/pests-diseases-weeds/animal/fmd
2. Holland, J., *et al*. (1982) Rapid evolution of RNA genomes. *Science* **215**, 1577–1585. doi:10.1126/science.7041255
3. Knowles, N.J. and Samuel, A.R. (2003) Molecular epidemiology of foot-and-mouth disease virus. *Virus Res.* **91**, 65–80. doi:10.1016/S0168-1702(02)00260-5
4. Paton, D.J., *et al*. (2009) Options for control of foot-and-mouth disease: knowledge, capability and policy. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **364**, 2657–2667. doi:10.1098/rstb.2009.0100
5. Condy, J.B., *et al*. (1969) Foot-and-mouth disease in wildlife in Rhodesia and other African territories. A serological survey. *J. Comp. Pathol.* **79**, 27–31. doi:10.1016/0021-9975(69)90023-1
6. Huang, C.C., *et al*. (2000) Characteristics of foot and mouth disease virus in Taiwan. *J. Vet. Med. Sci.* **62**, 677–679. doi:10.1292/jvms.62.677
7. Kitching, R.P. and Hughes, G.J. (2002) Clinical variation in foot and mouth disease: sheep and goats. *Rev. Sci. Tech.* **21**, 505–512.
8. Vosloo, W., *et al*. (2009) Longitudinal study to investigate the role of impala (*Aepyceros melampus*) in foot-and-mouth disease maintenance in the Kruger National Park, South Africa. *Transbound. Emerg. Dis* **56**, 18–30. doi:10.1111/j.1865-1682.2008.01059.x
9. Condy, J.B., *et al*. (1985) The duration of the foot-and-mouth disease virus carrier state in African buffalo (i) in the individual animal and (ii) in a free-living herd. *Comp. Immunol. Microbiol. Infect. Dis.* **8**, 259–265. doi:10.1016/0147-9571(85)90004-9
10. Bastos, A.D., *et al*. (2000) Natural transmission of foot-and-mouth disease virus between African buffalo (*Syncerus caffer*) and impala (*Aepyceros melampus*) in the Kruger National Park, South Africa. *Epidemiol. Infect.* **124**, 591–598. doi:10.1017/S0950268899004008
11. Vosloo, W., *et al*. (2002) The possible role that buffalo played in the recent outbreaks of foot-and-mouth disease in South Africa. *Ann. N. Y. Acad. Sci.* **969**, 187–190. doi:10.1111/j.1749-6632.2002.tb04376.x
12. Juleff, N., *et al*. (2008) Foot-and-mouth disease virus persists in the light zone of germinal centres. *PLoS ONE* **3**, e3434. doi:10.1371/journal.pone.0003434
13. Sutmoller, P. and Casas, O.R. (2002) Unapparent foot and mouth disease infection (sub-clinical infections and carriers): implications for control. *Rev. Sci. Tech.* **21**, 519–529.
14. Donaldson, A.I. and Alexandersen, S. (2002) Predicting the spread of foot and mouth disease by airborne virus. *Rev. Sci. Tech.* **21**, 569–575.
15. Thomson, G.R. and Bastos, A.D.S. (2004) Foot and mouth disease. In: *Infectious Diseases of Livestock* (Coetzer, J.A.W. and Tustin, R.C., eds), pp. 1324–1366, Oxford University Press.
16. Nasri, D., *et al*. (2007) Basic rationale, current methods and future directions for molecular typing of human enterovirus. *Expert Rev. Mol. Diagn.* **7**, 419–434. doi:10.1586/14737159.7.4.419
17. Tomasula, P.M., *et al*. (2007) Thermal inactivation of foot-and-mouth disease virus in milk using high-temperature, short-time pasteurization. *J. Dairy Sci.* **90**, 3202–3211. doi:10.3168/jds.2006-525
18. Paton, D.J., *et al*. (2010) Qualitative assessment of the commodity risk for spread of foot-and-mouth disease associated with international trade in deboned beef. *Transbound. Emerg. Dis.* **57**, 115–134. doi:10.1111/j.1865-1682.2010.01137.x
19. Thomson, G.R., *et al*. (2004) International trade in livestock and livestock products: the need for a commodity-based approach. *Vet. Rec.* **155**, 429–433.
20. http://www.animalhealthaustralia.com.au/wp-content/uploads/2011/04/FMD3_2-12-FINAL6May10.pdf
21. Mattion, N., *et al*. (2004) Reintroduction of foot-and-mouth disease in Argentina: characterisation of the isolates and development of tools for the control and eradication of the disease. *Vaccine* **22**, 4149–4162. doi:10.1016/j.vaccine.2004.06.040
22. Knowles, N.J., *et al*. (2009) Recent spread of a new strain (A-Iran-05) of foot-and-mouth disease virus type A in the Middle East. *Transbound. Emerg. Dis* **56**, 157–169. doi:10.1111/j.1865-1682.2009.01074.x
23. Lavoria, M.A., *et al*. (2012) Avidity and subtyping of specific antibodies applied to the indirect assessment of heterologous protection against Foot-and-Mouth Disease Virus in cattle. *Vaccine* **30**, 6845–6850. doi:10.1016/j.vaccine.2012.09.011
24. Eble, P.L., *et al*. (2006) Comparison of immune responses after intra-typic heterologous and homologous vaccination against foot-and-mouth disease virus infection in pigs. *Vaccine* **24**, 1274–1281. doi:10.1016/j.vaccine.2005.09.040
25. OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. (2012) http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.05_FMD.pdf
26. Reid, S.M., *et al*. (2001) Diagnosis of foot-and-mouth disease by real-time fluorogenic PCR assay. *Vet. Rec.* **149**, 621–623. doi:10.1136/vr.149.20.621
27. Brocchi, E., *et al*. (2006) Comparative evaluation of six ELISAs for the detection of antibodies to the non-structural proteins of foot-and-mouth disease virus. *Vaccine* **24**, 6966–6979. doi:10.1016/j.vaccine.2006.04.050

Biography

Wilna Vosloo has been working on FMD for over 20 years with vast experience in the disease, first gained in Africa and more recently in South East Asia. She is currently a senior research scientist at the Australian Animal Health Laboratory where she leads a major research project on FMD.

Aquatic animals; endemic and exotic bacterial pathogens

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The detection and identification of bacterial pathogens from fish and other aquatic animals presents particular challenges with regards to transportation of samples to the laboratory, knowledge of media and growth requirements, and detection of endemic or exotic pathogens. This article highlights the challenges in this specialised area of microbiology with a focus on an endemic and an exotic disease.

Bacteria from marine samples such as kingfish, prawn or seahorse, require media containing NaCl, but in addition to this some bacteria require magnesium and potassium ions. Bacteria from marine mammals such as whales and dolphins may need to be grown on media with and without salt as some organisms that are salt-requiring will adapt to normal physiological conditions in the marine mammal. In these cases initial growth may occur on media without salt, but for subsequent identification tests, NaCl must be added to the inoculum, as some reactions will give a false-negative result without NaCl despite the bacterium adapting to host conditions[1].

An incubation temperature of 25°C is generally used for aquatic samples, however some bacteria isolated from cold-water fish may have an optimal growth temperature of 18°C, whereas samples from marine mammals may grow best at 37°C. The best option is to incubate duplicate plates at both 25 and 37°C.

An endemic disease: the challenges of *Tenacibaculum maritimum*

Often fish farms are located huge distances from a laboratory and samples may not arrive in optimal condition to ensure isolation of the pathogen. An example is a fish farm in the far north of WA; a six hour boat trip to the nearest town with flights to Perth, means samples may take three days to reach the laboratory. When trying to isolate a slow-growing bacterium such as *Tenacibaculum maritimum*, sometimes the best option is inoculate culture plates at the farm (Figs 1, 2).

Tenacibaculum maritimum is the causative agent of tenacibaculosis, an ulcerative disease that affects a variety of marine fish species. Clinical signs include ulcers and necrosis on the body surface, eroded mouth, frayed fins and tail rot (Fig. 3)[2].

Tenacibaculum maritimum is an aerobic, oxidase positive, Gram-negative, slender, flexible rod $0.5 \times 2\,\mu m$ to $30\,\mu m$ with an occasional cell of $100\,\mu m$ in length. Cells have gliding motility on solid media[3]. Growth occurs on a low nutrient medium such as Anacker-Ordals medium supplemented with seawater or artificial sea salts (Fig. 4). Growth does not occur when supplemented with NaCl only, or on blood agar. Growth usually occurs on the commercially available MA 2216 agar (Difco)[2].

Figure 1. Fish farm off Kimberley coast, WA.

Figure 2. Collecting fish for sampling.

Figure 3. Barramundi displaying ulceration of skin and underlying musculature.

Figure 5. Yoyo loach infected with *E. ictaluri.*

Figure 4. *T. maritimum* on AO-marine agar at 4 d. Isolate from Dr Jeremy Carson, DPI WE, Tasmania.

Figure 6. Primary plate with *E. ictaluri* (arrows) in mixed culture, 72 h at 25°C on blood agar containing horse red blood cells.

Tenacibaculum maritimum does not ferment carbohydrates, is positive for congo red absorption and negative for production of flexirubin pigments as detected by KOH[2]. Conventional and real time PCRs have been reported for the detection and identification of *T. maritimum*[4,5].

An exotic disease: the not so innocent Nemo

The ornamental or aquarium fish trade in Australia is worth $350 M annually with 15.5 million fish being imported in 2005, and these imported fish are a potential source of exotic pathogens with potential to compromise Australian freshwater and marine aquaculture industries[6].

Fish imported into Australia are subject to Australian Quarantine and Inspection Service (AQIS) conditions and on arrival are held in an AQIS-approved quarantine premise for seven days, and if an exotic disease is detected, the consignment is destroyed. In Western Australia (WA), any fish showing signs of disease are sent to the Fish Health Unit, Department of Fisheries WA, and bacteriology is conducted at the Animal Health Laboratories, Department of Agriculture WA.

Edwardsiella ictaluri is the causative agent of enteric septicaemia of catfish (ESC). It is an important infectious disease in the commercial catfish industry in a number of countries including the USA, China, Thailand and Vietnam[7,8]. *E. ictaluri* is a potential pathogen of some non-ictalurids, as in experimental challenge of chinook salmon and rainbow trout mortality occurred at high doses[9].

E. ictaluri is exotic to Australia and a nationally notifiable disease. Since 1980 the organism has been reported a number of times from imported ornamental fish held in quarantine[10], and from quarantine facilities in three states in the past two years. Thus, it is important for Australian laboratories to be vigilant and to have the expertise to detect and identify this organism. A recent detection occurred in imported yoyo loach, so named for the markings on the body (Fig. 5). The remaining fish were destroyed to maintain Australia's disease-free status for this pathogen.

E. ictaluri is an oxidase-negative, Gram-negative rod to coccobacillus. Colonies on blood agar at 48 hours after incubation at 25–30°C are 1–2 mm, round, circular, slightly irregular and pale grey with a butyrous consistency (Fig. 6). It is Strep-like as a greenish-tinge may be seen around the colony and a weak beta-haemolysis underneath the colony. Growth occurs on nutrient agar, MacConkey agar and Salmonella-Shigella agar. Strains are motile at 25°C but non-motile at 37°C. *E. ictaluri* is not in the API 20E database and results will often give a percentage probability of *E. tarda*, a common pathogen. API 20E results for *E. ictaluri* have been reported as 400400057, 410400057 and 410414057 from catfish and rainbow trout isolates[1,8]. *E. ictaluri* is differentiated from *E. tarda* by being negative for indole and H_2S, whereas results are positive for *E. tarda*. As with all exotic pathogens, the identification is confirmed by the Australian Animal Health Laboratories at Geelong, Victoria.

A PCR has been reported for the specific detection of *E. ictaluri*[11].

References

1. Buller, N.B. (2004) *Bacteria from Fish and Other Aquatic Animals: a practical identification manual*, CABI.
2. Bernardet, J.-F., *et al*. (1994) Comparative study on *Flexibacter maritimus* strains isolated from farmed sea bass (*Dicentrarchus labrax*) in France. *Fish Pathol.* **29**, 105–111. doi:10.3147/jsfp.29.105
3. Wakabayashi, H., *et al*. (1986) *Flexibacter maritimus* sp. nov., a pathogen of marine fishes. *Int. J. Syst. Bacteriol.* **36**, 396–398. doi:10.1099/00207713-36-3-396
4. Bader, J.A. and Shotts, E.B. Jr (1998) Identification of *Flavobacterium* and *Flexibacter* species by species-specific polymerase chain reaction primers to the 16S ribosomal RNA gene. *J. Aquat. Anim. Health* **10**, 311–319. doi:10.1577/1548-8667(1998)010<0311:IOFAFS>2.0.CO;2
5. Fringuelli, E., *et al*. (2012) Development of a quantitative real-time PCR for the detection of *Tenacibaculum maritimum* and its application to field samples. *J. Fish Dis.* **35**, 579–590. doi:10.1111/j.1365-2761.2012.01377.x
6. Anon. (2006) A strategic approach to the management of ornamental fish in Australia, Natural Resource Management Ministerial Council. Department of Agriculture Fisheries and Forestry.
7. Hawke, J.P., *et al*. (1981) *Edwardsiella ictaluri* sp. nov., the causative agent of enteric septicaemia of catfish. *Int. J. Syst. Bacteriol.* **31**, 396–400. doi:10.1099/00207713-31-4-396
8. Crumlish, M., *et al*. (2002) Identification of *Edwardsiella ictaluri* from diseased catfish, *Pangasius hypophthalmus* (Sauvage), cultured in the Mekong Delta, Vietnam. *J. Fish Dis.* **25**, 733–736. doi:10.1046/j.1365-2761.2002.00412.x
9. Baxa, D.V., *et al*. (1990) Susceptibility of nonictalurid fishes to experimental infection with *Edwardsiella ictaluri*. *Dis. Aquat. Organ.* **8**, 113–117. doi:10.3354/dao008113
10. Humphrey, J.D., *et al*. (1986) Exotic bacterial pathogens *Edwardsiella tarda* and *Edwardsiella ictaluri* from imported ornamental fish *Betta splendens* and *Puntius conchonius*, respectively: isolation and quarantine significance. *Aust. Vet. J.* **63**, 369–371. doi:10.1111/j.1751-0813.1986.tb02900.x
11. Williams, M.L. and Lawrence, M.L. (2010) Verification of an *Edwardsiella ictaluri*-specific diagnostic PCR. *Lett. Appl. Microbiol.* **50**, 153–157. doi:10.1111/j.1472-765X.2009.02770.x

Biography

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Vets versus pets: methicillin-resistant *Staphylococcus aureus* in Australian animals and their doctors

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Humans and animals intimately sharing the same environment will inevitably be exposed to each other's microbiota. When one of those organisms is a drug-resistant pathogen then logistics of disease prevention are raised to a new level of complexity. For this reason the study of methicillin resistant *Staphylococcus aureus* (MRSA) in man and animals is now a priority. Recent research has demonstrated the ease with which MRSA crosses species barriers and the grave potential for MRSA to cause serious disease in animals and man has been well established. However, a key feature of MRSA (as compared zoonotic, resistant *Salmonella* spp.) is that companion and performance animals appear to have pivotal roles in the ecology of spread of certain genotypes found in humans. In this article we summarise the major developments in animal-human MRSA with an emphasis on the most recent Australian data incriminating involvement of companion and performance animals in the ecology of spread.

Staphylococcus aureus is responsible for a wide range of opportunistic infections in both humans and animals. In humans, infections with methicillin-resistant *S. aureus* (MRSA), which first appeared in the 1960s, have traditionally been nosocomial in origin. Hospital-associated MRSA strains cause serious and potentially fatal disease in patients with a wide range of predisposing conditions[1]. In the past 15 years new strains of MRSA have emerged that transmit between humans outside of health-care settings. These community associated MRSA are responsible for a growing burden of disease in otherwise healthy people in Australia[2] and abroad[1].

Until relatively recently, animals were not reported to play a major role in the transmission of MRSA to humans[3,4]. To date, MRSA has been identified in dogs, cats, pigs, sheep, poultry, horses, cattle, rabbits, seals, psittacine birds and other exotics including a bat, a turtle, a guinea pig and a chinchilla[5]. Internationally, MRSA has emerged as a significant and growing problem in small animal and

equine hospitals and intensive livestock facilities[6–9]. Surveys conducted in The Netherlands have shown MRSA prevalence in individual pigs on a single farm to be as high as 39%[10,11]. The strains colonising and causing infection in dogs and cats such as clonal-complex (CC) 22 most probably originated in humans but have not as yet become host-adapted in these companion animals[5,12]. By contrast, MRSA strains isolated from horses and livestock such as CC8 and ST398, which also originated in humans, have become adapted to their new hosts and are readily transmitted between individual animals. Strains of *Staphylococcus* normally associated with companion animals such as *Staphylococcus pseudintermedius* are also becoming resistant to methicillin, possibly via horizontal movement of *SCCmec* gene cassettes containing the *mecA* resistance gene into susceptible strains[13]. Internationally, veterinary personnel have much higher rates of MRSA nasal carriage compared to the general population and several cases of MRSA infection in humans have been attributed to close animal contact[14].

Australia is free of many animal diseases that are endemic in other countries thanks to strict enforcement of a robust quarantine policy, a ban on the importation of fresh meat and the absence of land borders with other countries[15]. To date, MRSA has not been reported in food-producing species in Australia. However, MRSA infections have been reported in companion animals in Australia, with the majority of strains belonging to CC22[4]. In addition, CC8 MRSA strains have been isolated from the nasal passage and occasionally, soft tissue infections in performance horses in New South Wales[16].

Given these existing parameters, we surveyed 771 Australian veterinarians attending various industry based conferences during the 2009 calendar year for MRSA nasal carriage (Table 1)[14]. Among the respondents, non-clinical veterinarians (who we regarded as our control population) had the lowest prevalence (0.9%). Veterinarians in mixed practice who indicated horses as a major area of work emphasis had a prevalence of 11.8% (13x the controls) and those who indicated that their major emphasis was only horses had a prevalence of 21.4% (23x the controls). Veterinarians with dogs and cats as a major activity had a 4.9% prevalence (5x the controls). These results confirm that animal contact in a clinical setting is an important risk factor for MRSA nasal carriage and highlight the need for better infection control, particularly in equine hospitals.

The CC identities of the 45 MRSA strains indicated that a high proportion of strains from companion animal veterinarians belonged to CC22 (76.9%). Many of these isolates showed resistance to ciprofloxacin whereas strains from equine practitioners belonged to CC8 (62.5%) and were more often resistant to gentamicin and rifampicin. One of the MRSA strains that was distinct from these CCs was isolated from a pig veterinarian with a recent history of international travel. This isolate has since been determined to belong to ST398[17]. While this result indicates that the major international animal-associated MRSA subtype ST398 does not appear to be prominent in Australia, more up to date studies are urgently required to determine how widespread it has become. Other major subtypes (CC22 and CC8) appear to be well established.

The prevalence of resistance to fluoroquinolones (used only in companion animals in Australia) was close to 100% in CC22 MRSA isolates sourced from veterinarians who worked exclusively with dogs and cats, but zero in isolates sourced from vets who worked exclusively with horses. Similarly, the prevalence of resistance to gentamicin and rifampin (used almost exclusively in horses) was much higher in isolates sourced from equine veterinarians compared with those who worked with dog and cats. As the resistance profiles of each respective CC closely match antibiotic usage patterns in each sector, this may indicate that the physical handling of antibiotics and administration to animals could be a significant risk factor for MRSA nasal carriage in veterinarians. Administration of antibiotics to animals can sometimes be a difficult and messy

Table 1. Results of the MRSA nasal swab survey undertaken for 771 Australian veterinarians attending conferences in 2009.

Conference	Number of swabs obtained	Number of web surveys completed	Number of swabs positive for MRSA	MRSA prevalence (%)
AVA[A]	477	461	14	2.94
APV[B]	29	27	1	3.45
ACVS Science Week[C]	168	158	11	6.55
Bain Fallon[D]	129	125	20	15.50
Total	803	771	46	5.73

[A]Australian Veterinary Association Annual Scientific Conference; [B]Australian Pig Veterinarians Conference; [C]Australian College of Veterinary Scientists Science; [D]Australian Equine Veterinarians Annual Scientific Conference.

process (for example administering rifampicin orally to foals with *Rhodococcus equi* infection).

Although there has been much progress in defining the ecology of MRSA in man and animals there remains considerable uncertainty. Enhanced surveillance and genetic analysis of the MRSA isolates so recovered would do much to address this. While on their own these activities do not lead to real-world progress they are, nevertheless, essential. For without such information it will be impossible to raise the importance of MRSA in the minds of those professionals (medical and veterinary) with the power to implement the infection control and prescribing practices needed to diminish the threat of MRSA.

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References

1. Bassetti, M., *et al*. (2009) Why is community-associated MRSA spreading across the world and how will it change clinical practice? *Int. J. Antimicrob. Agents* **34**, S15–S19. doi:10.1016/S0924-8579(09)70544-8
2. Nimmo, G.R. and Coombs, G.W. (2008) Community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) in Australia. *Int. J. Antimicrob. Agents* **31**, 401–410. doi:10.1016/j.ijantimicag.2007.08.011
3. Guardabassi, L., *et al*. (2004) Pet animals as reservoirs of antimicrobial-resistant bacteria. *J. Antimicrob. Chemother.* **54**, 321–332. doi:10.1093/jac/dkh332
4. Malik, S., *et al*. (2006) Molecular typing of methicillin-resistant staphylococci isolated from cats and dogs. *J. Antimicrob. Chemother.* **58**, 428–431. doi:10.1093/jac/dkl253
5. Morgan, M. (2008) Methicillin-resistant *Staphylococcus aureus* and animals: zoonosis or humanosis? *J. Antimicrob. Chemother.* **62**, 1181–1187. doi:10.1093/jac/dkn405
6. Baptiste, K.E., *et al*. (2005) Methicillin-resistant staphylococci in companion animals. *Emerg. Infect. Dis.* **11**, 1942–1944. doi:10.3201/eid1112.050241
7. Weese, J.S., *et al*. (2007) Cluster of methicillin-resistant *Staphylococcus aureus* colonization in a small animal intensive care unit. *J. Am. Vet. Med. Assoc.* **231**, 1361–1364. doi:10.2460/javma.231.9.1361
8. Cuny, C., *et al*. (2008) Clusters of infections in horses with MRSA ST1, ST254, and ST398 in a veterinary hospital. *Microb. Drug Resist.* **14**, 307–310. doi:10.1089/mdr.2008.0845
9. Van den Eede, A., *et al*. (2009) High occurrence of methicillin-resistant *Staphylococcus aureus* ST398 in equine nasal samples. *Vet. Microbiol.* **133**, 138–144. doi:10.1016/j.vetmic.2008.06.021
10. de Neeling, A.J., *et al*. (2007) High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. *Vet. Microbiol.* **122**, 366–372. doi:10.1016/j.vetmic.2007.01.027
11. van Duijkeren, E., *et al*. (2008) Transmission of methicillin-resistant *Staphylococcus aureus* strains between different kinds of pig farms. *Vet. Microbiol.* **126**, 383–389. doi:10.1016/j.vetmic.2007.07.021
12. Weese, J.S. and van Duijkeren, E. (2010) Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. *Vet. Microbiol.* **140**, 418–429. doi:10.1016/j.vetmic.2009.01.039
13. van Duijkeren, E., *et al*. (2008) Transmission of methicillin-resistant *Staphylococcus intermedius* between humans and animals. *Vet. Microbiol.* **128**, 213–215. doi:10.1016/j.vetmic.2007.11.016
14. Jordan, D., *et al*. (2011) Carriage of methicillin-resistant *Staphylococcus aureus* by veterinarians in Australia. *Aust. Vet. J.* **89**, 152–159. doi:10.1111/j.1751-0813.2011.00710.x
15. Animal Health Australia (2012) *Animal health in Australia 2011*. Animal Health Australia.
16. Axon, J.E., *et al*. (2011) Methicillin-resistant *Staphylococcus aureus* in a population of horses in Australia. *Aust. Vet. J.* **89**, 221–225. doi:10.1111/j.1751-0813.2011.00711.x
17. Trott, D. (2011) Public health risks of using antimicrobials in pigs. In *Manipulating Pig Production XIII*, pp. 174–176, Australasian Pig Science Association (Inc.).

Biographies

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Bacteriophage therapy

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Bacteriophages (phages) are viruses that infect only bacteria. They exhibit one of two types of life cycle; lytic (virulent) or lysogenic (temperate). They are non-toxic to other organisms, infecting, and in the case of lytic phages, multiplying rapidly within the bacterial host, ultimately killing it[1]. Lysogenic phages can remain in a quiescent state where the genome is integrated into the bacterial chromosome or exist as a plasmid. Some enhance bacterial virulence by encoding genes for toxins or antibiotic resistance[2]. Lytic phages are preferred for therapy as lysogenic phages may not result in host death and can transfer undesirable genes through transduction[1]. The history of prophylactic and therapeutic use of phages since their discovery over 90 years ago by d'Herelle (1917) and Twort (1915) are outlined in comprehensive reviews by Sulakvelidze *et al*, Merril and Hanlon[2–4]. Inconsistent and unreliable results combined with the discovery of antibiotics led to a decline in research in the West. The emphasis changed to the use of phages as tools for fundamental molecular studies focussing on the nature, replication and regulation of genes[5,6]. These studies clarified the biology of phages and provided a foundation for investigation into phage therapy and biocontrol.

Williams Smith and Huggins revived interest in phage therapy with their robust and repeatable evaluations of phages on a range of animal species with experimental *Escherichia coli* infections in the 1980s[7–9]. They demonstrated that a mixture of phages in a cocktail could significantly reduce morbidity and mortality due to toxigenic *E. coli* in calves, lambs and piglets. Importantly, protection against enteropathogenic *E. coli* challenge was afforded by phages present in the pens prior to admission and phage resistant variants were found to be less virulent[9].

By the 1990s it was increasingly evident that antibiotic control of pathogens was under threat from the prevalence of antibiotic resistant strains[10]. This combined with consumer and political pressure to reduce the use of antibiotics, especially in food production, led to a revival in interest in phage therapies as additional or alternative options. The prohibitive cost, time frame and stringent controls on drug development and trials in human medicine have meant that veterinary, agricultural and food safety applications have gained acceptance first. The kinetics of bacteriophage/bacterial host interaction is complex. Successful *in vivo* application requires analysis of the kinetic properties of each specific phage therapy[11]. Further to this, optimisation of formulation, delivery routes and long term stability data will be required[12]. The current pharmaceutical regulatory framework is not compatible with the dynamic nature of phages that do not fit comfortably within current therapeutic categories[13].

Phage based technologies currently used include typing of bacterial strains, as detection agents in ELISA based assays, food preparation/safety and the control of biofilms[14–16]. Table 1 is an overview of companies researching and producing phage based products.

Phage applications in food producing animals focus on prevention and treatment of infections or as potential biocontrol agents of zoonotic pathogens and directly on food surfaces. Those most frequently targeted have been *Campylobacter, Salmonella, Listeria* and *E. coli* O157:H7[17–20]. Significantly, in August 2006 the U.S Food and Drug Administration (FDA) approved under 'generally recognised as safe' (GRAS) regulations a phage cocktail as a spray for ready-to-eat meat to eradicate *Listeria monocytogenes*[21]. This was the first of a number of products proven and accepted to be effective and safe for human consumption and has been registered as an additive to organic foods in Europe and was approved for use as a food processing aid by Food Standards Australia & New Zealand (FSANZ) in Aug 2012[22].

Recent studies of therapeutic use targeting veterinary pathogens include oral administration of cocktails to calves & piglets against enterotoxigenic *E. coli*, topical treatment of *P. aeruginosa* otitis in dogs and oral, intratracheal or spray administration to treat colibacillosis in chickens[23–25]. Aquaculture is an industry where strategies for controlling infectious diseases are limited. Numerous studies of phage biocontrol have been carried out. Pathogens affecting a variety of fish species have been targeted including *Vibrio* species (vibriosis), *Photobacterium* (photobacteriosis), *Aeromonas*

Table 1. Companies involved in the development of phage-based products.

Company	Product	Target application
Detection/identification		
Microphage (USA)	MRSA/MSSA blood culture test	Bacteriophage amplification technology to identify the presence of viable pathogens and antibiotic susceptibility from clinical samples e.g. *Staphylococcus aureus* and methicillin, vancomycin and clindamycin susceptibility
Biophage Pharma Inc (Canada)	PDS® Biosensor BacTrapping In development	Phage based product for diagnosis of pathogens & toxins Phage based product for isolation and specific enrichment of bacteria from raw samples Phage bank for use in phage therapy applications and biosecurity e.g. *Bacillus anthracis*
Agriculture		
Omnilytics (USA)	AgriPhage™	Targets bacterial spot or speck on crops, with specific formulations for strains of *Xanthomonas campestris* pv. vesicatoria or *Pseudomonas syringae* pv. tomato
Aquaculture		
Phage Biotech Ltd (Israel)	In development	*Vibrio harveyi* water de-contaminant for shrimp hatcheries
Veterinary		
CheilJedang Corp. (Korea)	BioTector	Animal feed for control of *Salmonella gallinarum* (fowl typhoid) and *Salmonella pullorum* (pullorum disease) in poultry
Novolytics (UK)	In development	Treatments for MRSA in pigs and bovine mastitis
Biophage Pharma Inc (Canada)	Coli-Pro Salmo-Pro	Phages targeting enterotoxigenic *E. coli* in pigs and *Salmonella typhimurium* in chickens
AmpliPhi Biosciences Corp. (USA & UK)	In development	Phage cocktail targeting *Ps. aeruginosa* involved in canine ear infection being prepared for veterinary trials Phage mixture targeting *S. aureus* infections of bovine udders
Food safety		
Intralytix (USA)	EcoShield™ ListShield™	Targets *Escherichia coli* 0157:H7 contamination in foods and food-processing facilities Targets *Listeria monocytogenes* contamination in foods and food-processing facilities
Micreos (The Netherlands)	LISTEX™ P100 SALMONELEX™ undergoing field trials	Targets *Listeria monocytogenes* strains on food products Targets *Salmonella* on food products

Table 1. (*continued*)

Company	Product	Target application
Therapeutic		
Novolytics (UK)	In development – gels for targeting MRSA and *Clostridium difficile*	Gel containing a cocktail of phages targeted at MRSA to treat nasal carriage of MRSA Gels for skin infections and indwelling medical devices
GangaGen Inc (India)	In development	Proprietary recombinant protein based on a phage muralytic enzyme for the topical prevention and treatment of Staphylococcal infections, including MRSA
Phico Therapeutics (UK)	Delivery of small acid-soluble spore protein (SASP) genes to target bacteria using modified bacteriophage vectors	SASPject™ PT1.2: early clinical development aimed at *S. aureus* including MRSA SASPject™ PT3.1: pre-clinical development for systemic use against *Ps. aeruginosa* SASPject™ PT3.X for systemic use against Gram -ve bacteria e.g. *E. coli* and *K. pneumoniae*.
AmpliPhi Biosciences Corp. (USA and UK)	BioPhage-PA – development for Phase 3 trials BioPhage-PR – being prepared for clinical trials In development	Phage treatment against *Pseudomonas aeruginosa* in chronic ear disease and topical infections Phage treatment against *Pseudomonas aeruginosa* associated with cystic fibrosis Other targets include *A. baumannii*, *S. maltophilia*, *S. aureus*, *C. difficile*.

salmonicida (furunculosis) and *Flavobacterium psychrophilum* (bacterial cold water disease)[26–28].

Therapeutic and prophylactic phage treatments do have limitations. Studies have noted that phage treatments often result in transient reduction not elimination of the target pathogens[29]. This is a likely consequence of the dynamic nature of the phage host relationship leading to coexistence of the phage and host and phage-resistant sub-populations. It is recommended that for prevention of food borne pathogens phages should be administered just prior to transport or slaughter to maximise pathogen load reduction and minimise resistance[30,31]. Prophylactic use of phages in intensive production facilities has risks associated with potential selection of resistant strains. Restricting use to therapeutic application to limit dissemination of phages into the natural environment is recommended[32].

In vitro and clinical investigations of combination therapies with antibiotics have been shown to reduce selection of phage resistant variants and facilitate the spread of phages by enhancing lysis of the host bacterial cells[33–35]. Phage-antibiotic synergy (PAS) has the potential to reduce the amount of antibiotics administered[34].

Phages are released from the host cell through the action of two phage genome encoded proteins - holins disrupt the cell membrane and endolysins digest the cell wall[36]. Endolysins are also able to lyse gram positive bacteria when applied exogenously. Advantages over antibiotic or phage therapy include reduced likelihood of resistance, endolysin specificity, the potential for genetic modification and the fact that they can be sourced from lytic or lysogenic phages[37–39].

The host specificity of phages based on interaction with bacterial surface receptors has been exploited in a number of novel technologies for targeted gene, drug and vaccine delivery[40,41]. Phage tail-spike proteins (Tsp) mediate specificity through interaction with host receptors. Many have endoglycosidase activity leading to the hydrolysis of the receptor that has been shown to interfere with bacterial cell motility, significantly reducing colonisation and invasiveness[42]. Advantages are a reduction in resistance of target cells and no release of potentially toxic bacterial cell components during lysis.

A novel approach is the reversal of antibiotic resistance of pathogens using lysogenic phages to introduce genes conferring sensitivity to resistant strains[43].

Phages and phage-based products are not the panacea to control bacterial infections or food borne diseases however when carefully evaluated and in combination with other strategies they have tremendous potential.

References

1. Kutter, E. and Sulakvelidze, A. (2005) eds. *Bacteriophages Biology and Applications*. 2005, CRC Press: Florida. 510.
2. Merril, C., *et al*. (2003) The prospect for bacteriophage therapy in Western medicine. *Nat. Rev. Drug Discov.* **2**, 489–497. doi:10.1038/nrd1111
3. Sulakvelidze, A., *et al*. (2001) Bacteriophage therapy. *Antimicrob. Agents Chemother.* **45**, 649–659. doi:10.1128/AAC.45.3.649-659.2001
4. Hanlon, G.W. (2007) Bacteriophages: an appraisal of their role in the treatment of bacterial infections. *Int. J. Antimicrob. Agents* **30**, 118–128. doi:10.1016/j.ijantimicag.2007.04.006
5. Ellis, E.L. and Delbruck, M. (1939) The growth of bacteriophage. *J. Gen. Physiol.* **22**, 365–384. doi:10.1085/jgp.22.3.365
6. Lwoff, A. (1953) Lysogeny. *Bacteriol. Rev.* **17**, 269–337.
7. Williams Smith, H. and Huggins, M.B. (1983) Effectiveness of phages in treating experimental *Escherichia coli* diarhoea in calves, piglets and lambs. *J. Gen. Microbiol.* **129**, 2659–2675.
8. Williams Smith, H., *et al*. (1987) Factors influencing the survival and multiplication of bacteriophages in calves and in their environment. *J. Gen. Microbiol.* **133**, 1127–1135.
9. Williams Smith, H., *et al*. (1987) The control of experimental *Escherichia coli* diarrhoea in calves by means of bacteriophages. *J. Gen. Microbiol.* **133**, 1111–1126.
10. French, G.L. (2010) The continuing crisis in antibiotic resistance. *Int. J. Antimicrob. Agents* **36**, S3–S7. doi:10.1016/S0924-8579(10)70003-0
11. Cairns, B.J., *et al*. (2009) Quantitative models of *in vitro* bacteriophage-host dynamics and their application to phage therapy. *PLoS Pathog.* **5**, e1000253.
12. Ryan, E.M., *et al*. (2011) Recent advances in bacteriophage therapy: How delivery routes, formulation, concentration and timing influence the success of phage therapy. *J. Pharm. Pharmacol.* **63**, 1253–1264. doi:10.1111/j.2042-7158.2011.01324.x
13. Pirnay, J.P., *et al*. (2012) Introducing yesterday's phage therapy in today's medicine. *Future Virol.* **7**, 379–390. doi:10.2217/fvl.12.24
14. Hagens, S. and Loessner, M. (2007) Application of bacteriophages for detection and control of foodborne pathogens. *Appl. Microbiol. Biotechnol.* **76**, 513–519. doi:10.1007/s00253-007-1031-8
15. Carson, L., *et al*. (2010) The use of lytic bacteriophages in the prevention and eradication of biofilms of *Proteus mirabilis* and *Escherichia coli*. *FEMS Immunol. Med. Microbiol.* **59**, 447–455.
16. Chibeu, A., *et al*. (2012) Bacteriophages with the ability to degrade uropathogenic *Escherichia coli* biofilms. *Viruses* **4**, 471–487. doi:10.3390/v4040471
17. Abuladze, T., *et al*. (2008) Bacteriophages reduce experimental contamination of hard surfaces, tomato, spinach, broccoli, and ground beef with *Escherichia coli O157:H7*. *Appl. Environ. Microbiol.* **74**, 6230–6238. doi:10.1128/AEM.01465-08
18. Bardina, C., *et al*. (2012) Significance of the bacteriophage treatment schedule in reducing salmonella colonization of poultry. *Appl. Environ. Microbiol.* **78**, 6600–6607. doi:10.1128/AEM.01257-12
19. Bigot, B., *et al*. (2011) Control of *Listeria monocytogenes* growth in a ready-to-eat poultry product using a bacteriophage. *Food Microbiol.* **28**, 1448–1452. doi:10.1016/j.fm.2011.07.001
20. Carvalho, C.M., *et al*. (2010) The *in vivo* efficacy of two administration routes of a phage cocktail to reduce numbers of *Campylobacter coli* and *Campylobacter jejuni* in chickens. *BMC Microbiol.* **10**, 232. doi:10.1186/1471-2180-10-232
21. FDA (2006) *Food and Drugs Title 21, Volume 3, Subchapter B - Food for Human Consumption*, in *Chapter I*.
22. FSANZ, F.S.A.N.Z., (2012) *Approval Report – Application A1045* in *Bacteriophage Preparation P100 as Processing Aid*.
23. Jamalludeen, N., *et al*. (2009) Evaluation of bacteriophages for prevention and treatment of diarrhea due to experimental enterotoxigenic *Escherichia coli* O149 infection of pigs. *Vet. Microbiol.* **136**, 135–141. doi:10.1016/j.vetmic.2008.10.021
24. Hawkins, C., *et al*. (2010) Topical treatment of *Pseudomonas aeruginosa* otitis of dogs with a bacteriophage mixture: A before/after clinical trial. *Vet. Microbiol.* **146**, 309–313. doi:10.1016/j.vetmic.2010.05.014
25. Oliveira, A., *et al*. (2010) *In vivo* efficiency evaluation of a phage cocktail in controlling severe colibacillosis in confined conditions and experimental poultry houses. *Vet. Microbiol.* **146**, 303–308. doi:10.1016/j.vetmic.2010.05.015
26. Stenholm, A.R., *et al*. (2008) Isolation and characterization of bacteriophages infecting the fish pathogen *Flavobacterium psychrophilum*. *Appl. Environ. Microbiol.* **74**, 4070–4078. doi:10.1128/AEM.00428-08
27. Defoirdt, T., *et al*. (2011) Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Curr. Opin. Microbiol.* **14**, 251–258. doi:10.1016/j.mib.2011.03.004
28. Oliveira, J., *et al*. (2012) Bacteriophage therapy as a bacterial control strategy in aquaculture. *Aquacult. Int.* **20**, 879–910. doi:10.1007/s10499-012-9515-7
29. Sheng, H., *et al*. (2006) Application of bacteriophages to control intestinal *Escherichia coli* O157:H7 levels in ruminants. *Appl. Environ. Microbiol.* **72**, 5359–5366. doi:10.1128/AEM.00099-06
30. Wall, S.K., *et al*. (2010) Phage therapy to reduce preprocessing Salmonella infections in market-weight swine. *Appl. Environ. Microbiol.* **76**, 48–53. doi:10.1128/AEM.00785-09
31. Doyle, M.P. and Erickson, M.C. (2012) Opportunities for mitigating pathogen contamination during on-farm food production. *Int. J. Food Microbiol.* **152**, 54–74. doi:10.1016/j.ijfoodmicro.2011.02.037
32. Sillankorva, S., *et al*. (2010) *Salmonella* Enteritidis bacteriophage candidates for phage therapy of poultry. *J. Appl. Microbiol.* **108**, 1175–1186. doi:10.1111/j.1365-2672.2009.04549.x
33. Verma, V., *et al*. (2009) Restricting ciprofloxacin-induced resistant variant formation in biofilm of *Klebsiella pneumoniae* B5055 by complementary bacteriophage treatment. *J. Antimicrob. Chemother.* **64**, 1212–1218. doi:10.1093/jac/dkp360
34. Comeau, A.M., *et al*. (2007) Phage-antibiotic synergy (PAS): β-lactam and quinolone antibiotics stimulate virulent phage growth. *PLoS ONE* **2**, e799. doi:10.1371/journal.pone.0000799
35. Kutateladze, M. and Adamia, R. (2010) Bacteriophages as potential new therapeutics to replace or supplement antibiotics. *Trends Biotechnol.* **28**, 591–595. doi:10.1016/j.tibtech.2010.08.001
36. Hermoso, J.A., *et al*. (2007) Taking aim on bacterial pathogens: from phage therapy to enzybiotics. *Curr. Opin. Microbiol.* **10**, 461–472. doi:10.1016/j.mib.2007.08.002
37. O'Flaherty, S., *et al*. (2009) Bacteriophage and their lysins for elimination of infectious bacteria. *FEMS Microbiol. Rev.* **33**, 801–819. doi:10.1111/j.1574-6976.2009.00176.x
38. Fischetti, V.A. (2010) Bacteriophage endolysins: A novel anti-infective to control Gram-positive pathogens. *Int. J. Med. Microbiol.* **300**, 357–362. doi:10.1016/j.ijmm.2010.04.002
39. Borysowski, J., *et al*. (2011) Potential of bacteriophages and their lysins in the treatment of MRSA: Current status and future perspectives. *BioDrugs* **25**, 347–355. doi:10.2165/11595610-000000000-00000
40. Vaks, L. and Benhar, I. I. (2011) Antibacterial application of engineered bacteriophage nanomedicines: antibody-targeted, chloramphenicol prodrug loaded bacteriophages for inhibiting the growth of *Staphylococcus aureus* bacteria, in *Methods in molecular biology* S.J. Hurst, Editor. Springer Science+Business Media: Clifton, N.J. p. 187–206.
41. Clark, J.R., *et al*. (2011) Comparison of a bacteriophage-delivered DNA vaccine and a commercially available recombinant protein vaccine against hepatitis B. *FEMS Immunol. Med. Microbiol.* **61**, 197–204. doi:10.1111/j.1574-695X.2010.00763.x
42. Waseh, S., *et al*. (2010) Orally administered P22 phage tailspike protein reduces *Salmonella* colonization in chickens: Prospects of a novel therapy against bacterial infections. *PLoS ONE* **5**, e13904. doi:10.1371/journal.pone.0013904
43. Edgar, R., *et al*. (2012) Reversing bacterial resistance to antibiotics by phage-mediated delivery of dominant sensitive genes. *Appl. Environ. Microbiol.* **78**, 744–751. doi:10.1128/AEM.05741-11

Biography

Dr Kate Hodgson has many years experience working in microbiology in industry and research laboratories in Adelaide, Canberra and London. She has also taught Microbiology in the School of Pharmacy and Medical Sciences at the University of South Australia. Her PhD research involved the isolation and characterisation of bacteriophages specific for enterotoxigenic *Escherichia coli* associated with post-weaning diarrhoea in piglets.

An update on the MLST scheme for *Pasteurella multocida*

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Pasteurella multocida **is a cause of economically important diseases in almost all domestic livestock species, as well as wildlife. While a range of typing methods have traditionally been used, the development of a Multi-Locus Sequence Typing (MLST) scheme in 2010 represented the first standardised, sequence based, Web supported typing scheme. The initial scheme (termed the RIRDC MLST scheme) was based on 63 avian isolates from diseased Australian poultry and three international reference strains, which formed 29 Sequence Types (STs). The MLST database (http://pubmlst.org/pmultocida_rirdc/) now contains data from over 560 isolates that form 220 STs. The use of the scheme in published studies to date has demonstrated some key points: A) the highly clonal nature of haemorrhagic septicaemia (HS) isolates; B) bovine respiratory isolates are typically very distinct from HS isolates; C) evidence of host/niche association (i.e. some STs are associated with specific hosts); and D) the distinct genotype of *P. multocida* isolates of capsule type B from calf pleuritis and peritonitis cases in New Zealand. The continued use of this MLST scheme by research groups around the world will add to our understanding of the population structure and host associations of this major veterinary pathogen.**

Pasteurella multocida is an iconic bacterium that has links back to the beginnings of veterinary microbiology with Louis Pasteur and his pioneering work on a vaccine based on an attenuated strain[1]. The bacterium is a Gram negative coccobacillus that is associated with a range of diseases in wild and domestic animals and is also part of the normal oropharyngeal flora of these animals[2]. There are many published typing schemes, both traditional methods such as serotyping and phenotyping, as well as molecular based methods[3]. Until recently, the generally agreed "gold standard" typing method of Multi-locus Sequence Typing (MLST)[4] had not been applied to *P. multocida*. A recent Australian study, completed in 2010[5], developed the first Web-supported MLST for *P. multocida*. This article looks at that initial publication and reviews developments since that initial publication.

Initial development of an MLST scheme

The development of an MLST scheme for *P. multocida* was made easier by the existence of an earlier multi-locus enzyme electrophoresis (MLEE) scheme for *P. multocida*[6]. MLEE schemes were a phenotypic precursor of MLST schemes and were based on differences in amino acid sequences in enzymes detected by starch gel electrophoresis[6]. While cumbersome, the *P. multocida* MLEE scheme generated novel information – the first evidence that the avian *P. multocida* population consisted of two quite different clusters[6] that did not match the earlier proposal of three subspecies – subspecies *multocida, gallicida* and *septica* – based on DNA/DNA hybridization data[7]. As the earlier MLEE scheme and the RIRDC MLST scheme were based on the same house-keeping enzymes, it is not surprising that the initial RIRDC MLST scheme (based on 63 Australian avian isolates) recognised the same two major clusters within avian *P. multocida*[6]. Furthermore, retrospective application of the MLST to a well characterised set of *P. multocida* isolates from eight fowl cholera outbreaks showed that the RIRDC MLST scheme provided epidemiologically relevant typing results that matched those provided by a range of other genotypic methods[6].

Subsequent use of the RIRDC MLST Scheme

As a result of both formally published studies and addition of MLST results from unpublished studies, the current RIRDC *P. multocida* MLST scheme contains 560 *P. multocida* isolates in 220 STs. The isolates have come from 41 different hosts (including both domestic livestock and wildlife). The largest number of isolates are from cattle (around 25% of isolates) with two other domestic livestock species well represented (pigs at 17% and chickens at 16%). Isolates from wild animals include those from chimpanzees (four isolates), tigers (two isolates) and elephants (two isolates).

The largest single published study using the RIRDC MLST database has resulted from the work of the Moredun Research Institute,

which examined 201 *P. multocida* isolates, 128 of which were bovine respiratory isolates from the UK, France and the USA[8]. The Moredun study found that the bovine respiratory disease isolates were clonal, with 105 isolates belonging to a single clonal complex (CC13)[8]. These respiratory isolates were quite distinct from haemorrhagic septicaemia (HS) isolates[8]. The clonal nature of HS isolates is confirmed within the complete database, with 46 of 49 HS isolates allocated to a single ST – ST122. The other key finding from the Moredun study was the finding that 58 of the 62 STs detected in the study were associated with only one host type[8].

Perhaps the most unusual use of the RIRDC MLST scheme to date has been the examination of four *P. multocida* isolates from wild chimpanzees in Côte D'Ivoire that were associated with two severe respiratory disease outbreaks in the same national park in 2004 and 2009[9]. The MLST results showed that two genotypes were involved with the 2004 outbreak, with one of these genotypes re-appearing in the 2009 outbreak, findings also supported by other typing methods.

In another example of the power of MLST, a New Zealand study examined very unusual outbreaks of calf peritonitis and pleuritis associated with *P. multocida* of capsular type B[10]. Until this report, the accepted paradigm was that capsule type B was always associated with HS outbreaks. The use of the RIRDC MLST scheme showed that these atypical serovar B types did not belong to the clone associated with HS (ST122), but rather to a unique ST not previously reported[10].

Future

There is no doubt that our understanding of the population structure and the global epidemiology of *P. multocida* will increase as more use is made of an array of new and emerging technologies. The RIRDC MLST scheme will be a key plank that helps provide an overarching view of the population structure and possible host associations, particularly as more data are added.

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The research to develop the RIRDC MLST scheme was part of the unfinished M. Phil. research work of Sounthi Subaaharan. Sounthi passed away shortly after the publication of the RIRDC MLST scheme after a gallant battle against illness. The RIRDC MLST scheme remains a living tribute to her skills and expertise. The funding of the Chicken Meat R & D Committee within the Rural Industries Research and Development Corporation (RIRDC) was a crucial part of our work. The establishment of the RIRDC MLST scheme was supported by Keith Jolley and is sited at the University of Oxford[11].

References

1. Glisson, J.R. *et al*. (2008) Fowl Cholera. In *Diseases of Poultry* (Saif, Y. M. *et al.* eds), pp 739–758. Blackwell Publishing.
2. Boyce, J.D. *et al*. (2010) *Pasteurella.* In *Pathogenesis of Bacterial Infections in Animals* (Gyles, C. L. *et al.* eds), pp 325–346, Wiley Blackwell.
3. Blackall, P.J., *et al*. (2000) Identification and typing of *Pasteurella multocida*: a review. *Avian Pathol.* **29**, 271–287. doi:10.1080/03079450050118395
4. Maiden, M.C., *et al*. (1998) Multi-locus sequence typing: a portable approach to the identification of clones within populations of pathogenic micro-organisms. *Proc. Natl. Acad. Sci. USA* **95**, 3140–3145. doi:10.1073/pnas.95.6.3140
5. Subaaharan, S., *et al*. (2010) Development of a multi-locus sequence typing scheme for avian isolates of *Pasteurella multocida*. *Vet. Microbiol.* **141**, 354–361. doi:10.1016/j.vetmic.2010.01.017
6. Blackall, P.J., *et al*. (1998) Population structure and diversity of avian isolates of *Pasteurella multocida* from Australian poultry. *Avian Pathol.* **144**, 279–288.
7. Mutters, R., *et al*. (1985) Reclassification of the genus *Pasteurella* Trevisan 1887 on the basis of deoxyribonucleic acid homology, with proposals for the new species *Pasteurella dagmatis, Pasteurella canis, Pasteurella stomatis, Pasteurella antatis* and *Pasteurella langaa*. *Int. J. Syst. Bacteriol.* **35**, 309–322. doi:10.1099/00207713-35-3-309
8. Hotchkiss, E.J., *et al*. (2011) Multilocus sequence typing of a global collection of *Pasteurella multocida* isolates from cattle and other host species demonstrates niche association. *BMC Microbiol.* **11**, 115. doi:10.1186/1471-2180-11-115
9. Köndgen, S., *et al*. (2011) *Pasteurella multocida* involved respiratory disease of wild chimpanzees. *PLoS ONE* **6**, e24236. doi:10.1371/journal.pone.0024236
10. McFadden, A.M.J., *et al*. (2011) Outbreaks of pleuritis and peritonitis in calves associated with *Pasteurella multocida* capsular type B strain. *N. Z. Vet. J.* **59**, 40–45. doi:10.1080/00480169.2011.547168
11. Jolley, K.A., *et al*. (2004) mlstdbNet – distributed multilocus sequence typing (MLST) databases. *BMC Bioinform.* **5**, 86. doi:10.1186/1471-2105-5-86

Biography

Pat Blackall is the Research Manager of the Poultry CRC as well as a Principal Research Fellow within the Queensland Alliance for Agriculture and Food Innovation within the University of Queensland. His research interests cover bacterial respiratory diseases of intensively raised livestock, molecular epidemiology, antimicrobial resistance, rapid identification and typing of bacterial pathogens and on-farm aspects of food safety.

Intestinal Spirochaetes and Brachyspiral colitis

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The "intestinal spirochaetes" are a group of anaerobic bacteria assigned to various species in the genus *Brachyspira*. They inhabit the large intestines of birds and animals – but also may be found in human beings. These bacteria first came to prominence in the early 1970s when a spirochaete named *Treponema hyodysenteriae* (now *Brachyspira hyodysenteriae*) was shown to be the agent of swine dysentery, a colonic infection of pigs that is endemic in many countries and is of considerable economic significance. Since the initial description, related spirochaetes have been identified and characterised and various name changes have occurred – finally resulting in the genus *Brachyspira* and its seven officially recognised species. Many different hosts are colonised with the various *Brachyspira* species, but disease is mainly reported in pigs and in adult chickens. Humans are colonised with the zoonotic *Brachyspira pilosicoli* and *Brachyspira aalborgi*. Reduced susceptibility to various antimicrobials is now starting to represent a major problem for effective control of Brachyspiral colitis in pigs and other species, and consequently attention is focusing on the development of new vaccines. The *Brachyspira* species have specialised growth requirements, and different species can take from three days to three weeks to form a thin film of visible growth on selective isolation plates. Genetic manipulation of individual strains remains difficult, and this has limited understanding of gene function and disease pathogenesis. Recently whole genomic sequencing projects have started to reveal much that was previously unknown about these specialised bacteria.

Taxonomy and disease association

Currently the genus *Brachyspira* includes seven officially named species and a variety of unofficially proposed members. Some species have well-established pathogenic potential in certain hosts, causing forms of *Brachyspira* colitis, whilst others are considered to be largely commensal (see Table 1). The *Brachyspira* are genetically distinct from other spirochaetes, and the close similarities between some of the species in their 16S rRNA sequences suggest that speciation in the genus has occurred relatively recently and rapidly. Apart from the strength of haemolysis, there are few clear phenotypic differences between the species, and indeed the boundaries between some of the named species are indistinct both genetically and phenotypically.

Population structures, evolution and genetic variation

For some species, such as *B. hyodysenteriae*, studies using multilocus sequence typing have provided clear evidence of the population structure being clonal[1,2], while for *B. pilosicoli* the population appears to be recombinant[3]. In the case of *B. hyodysenteriae*, the adaptation to a lifestyle in the large intestine appears to have included acquisition of various genes from *Escherichia coli* and *Clostridium* species, especially those encoding proteins associated with transport and metabolism[4]. These are likely to have been acquired in the densely populated, complex and specialised environment of the large intestine.

The existence of extensive genetic rearrangements can be observed within and between *Brachyspira* species, with sequence drift also generating diversity. The variation and fluidity of the genomes can be seen in the case of *B. pilosicoli*, where three sequenced strains had genome sizes of ~2,765, 2.890 and 2.596 Mb respectively[5], with genome rearrangements that largely correlated with the positions of mobile genetic elements. Novel bacteriophages were detected, as they were in a previous genomic study on *B. intermedia*[6]. These bacteriophages, that have themselves undergone extensive gene remodelling, are involved in intra- and inter-species horizontal gene transfer, and are likely to be a major force in the evolution of the *Brachyspira* species. In addition, novel genetic information may be acquired through the activity of prophage-like gene transfer agents that are present in the genome of different *Brachyspira* species[7,8]. Evidence for rapid genetic change can be seen at the farm level, where, for example, "microevolution" of *B. hyodysenteriae* strains resulting in changed DNA profiles has been recorded over relatively short time periods[9,10].

Table 1. *Brachyspira* species, their hosts and disease associations.

Species name	Haemolysis on blood agar	Main host species	Pathogenic potential, and other comments
B. hyodysenteriae	Strong	Pigs, occasionally poultry	The agent of swine dysentery, a severe mucohaemorrhagic colitis. Strains of varying virulence have been described.
"*B. suanatina*"	Strong	Mallards, pigs	Pathogenic in pigs experimentally. Only described in Scandinavia to date.
"*B. hampsonii*"	Strong	Pigs	Recently emerged as a cause of swine dysentery-like disease in North America. Positive in *B. intermedia* PCR, but genetically distinct.
B. pilosicoli	Weak	Pigs, poultry, humans and many other species	An agent of intestinal spirochaetosis in pigs, poultry, humans and other species. Characterised by end-on attachment to colonic enterocytes, with colitis and diarrhoea. Spirochaetaemia has been recorded in debilitated human beings and there is recent evidence of systemic spread in chickens[21].
B. alvinipulli	Weak	Chickens, other poultry	An agent of avian intestinal spirochaetosis (AIS).
B. intermedia	Weak	Chickens, pigs	A common pathogen in adult chickens (causing AIS). Strains occasionally are associated with diarrhoea in pigs. This is a genetically diverse group that may include several species.
B. murdochii	Weak	Pigs, chickens	Occasionally associated with mild colitis in pigs.
B. innocens	Weak	Pigs	Generally considered to be non-pathogenic, but occasionally associated with diarrhoea.
B. aalborgi	Weak	Humans	Uncertain clinical significance. Attached by one cell end to colonic enterocytes.
Various other proposed species	Weak	Various – include birds, dogs and rodents	Include "*B. canis*", "*B. pulli*", "*B. corvi*", "*B. rattus*" and "*B. muris*". Probably commensals.

Pathogenesis

The basis of virulence in the various *Brachyspira* species is still imperfectly understood. In order for pathogenic *Brachyspira* species to induce disease it is essential for them to colonise the large intestine and to grow to large numbers. Their anaerobic metabolism and use of substrates has been fine tuned to allow them to thrive in the milieu of the large intestine. There are complex physical and chemical interactions that occur between components of the diet and the normal colonic microbiota: these profoundly influence the environment, and it has become clear that the resultant conditions can affect colonisation by the spirochaetes[11].

As part of the colonisation process *Brachyspira* cells must move through the mucus overlying the epithelium of the large intestine. The corkscrew-like motility of *B. hyodysenteriae* has long been known to be an important virulence attribute, allowing it to penetrate the mucus. In the case of *B. pilosicoli,* this spirochaete shows increased motility under viscous conditions[12], including mucin concentrations equivalent to those found in the colon[13]. In addition to their motility, the cells of different *Brachyspira* species demonstrate a chemotactic attraction to colonic mucin. Comparison of the genome sequences of *B. hyodysenteriae* and *B. pilosicoli* has shown that *B. pilosicoli* has fewer methyl-accepting chemotaxis genes than *B. hyodysenteriae,* and completely lacks *mcpC* genes; hence these species are predicted to have different chemotactic responses, and this in turn may help to explain their different host ranges and colonisation sites in the large intestine[14]. Experimentally, although cells of both *B. hyodysenteriae* and *B. pilosicoli* are attracted to and enter mucin solutions, this was reduced at mucin concentrations above 6% for *B. hyodysenteriae* but not for *B. pilosicoli*[13], again providing a possible explanation for their different colonisation sites.

A likely virulence determinant in *B. hyodysenteriae* is the strong haemolytic activity of this spirochaete. This is supported by the fact that two other recently described strongly haemolytic species are also pathogenic[15,16] (see Table 1). Currently eight genes encoding proteins with predicted haemolytic activity have been described in *B. hyodysenteriae*[4,14], but their respective roles have not all been confirmed experimentally.

A recent *in vitro* study using Caco-2 cell monolayers has provided some insights into how *B. pilosicoli* interacts with colonic enterocytes to cause disease[17], and similar detailed studies are required with *B. hyodysenteriae* and other species. In that study[17] the Caco-2 cell junctions were shown to be the initial targets of the characteristic end-on attachment by *B. pilosicoli.* Colonised monolayers then demonstrated a time-dependent series of changes, including accumulation of actin at the cell junctions, loss of tight junction integrity and condensation and fragmentation of nuclear material consistent with the occurrence of apoptosis. The colonised monolayers demonstrated a significant up-regulation of interleukin-1ß (IL-1ß) and IL-8 expression. These cytokines/chemokines are likely to be responsible for attracting inflammatory cells to the colonisation site, and causing localised colitis. Potential mechanisms for inducing such cellular damage include the biological activity of lipooligosaccharides and/or the action of membrane proteases.

Sequencing of the genome of *B. hyodysenteriae* strain WA1 resulted in the identification of a previously unrecognised plasmid that contained 31 genes, including six *rfbA-D* genes that were predicted to be involved with rhamnose biosynthesis, and hence LOS structure, as well as others associated with glycosylation[4]. Subsequently avirulent strain A1 was shown to lack the plasmid, and when an Australian field isolate lacking the plasmid was selected and used experimentally to infect pigs, significantly fewer became colonised and developed dysentery compared to the pigs infected with a control strain that contained the plasmid[18]. The results support the likelihood that plasmid-encoded genes of *B. hyodysenteriae* are involved in colonisation and/or in disease expression.

Recombinant vaccines

Recently recombinant protein vaccines have received attention as potential vaccine components for *Brachyspira* species: for example, vaccination with recombinant outer-membrane lipoprotein Bhlp29.7 from *B. hyodysenteriae* provided a 50% reduction in the incidence of disease compared to unvaccinated controls following experimental infection[19]. The availability of genome sequences has provided the opportunity to broaden this approach through the application of "reverse vaccinology", where scores of such predicted proteins can be identified from the genome sequence, screened and tested as vaccine candidates. This approach has been used successfully with *B. hyodysenteriae*[20], and it is anticipated that a new generation of commercial vaccines based on this approach will become available in the next few years.

Summary

With the recent availability of *Brachyspira* genome sequences and new technologies better insights into the growth requirements and pathogenic mechanisms of *Brachyspira* species are emerging. This information is of direct benefit for control of the infections, since, for example, information about growth and colonisation requirements derived from metabolic reconstructions of the spirochaetes can help to predict what changes in the colonic environment are likely to reduce their growth[4,14]. Further detailed studies are needed to determine how the colonic microbiota is influenced by different dietary substrates, and how this impacts on colonisation by *Brachyspira* species. The sequence data has also allowed the use of a reverse vaccinology approach to vaccine development.

References

1. La, T., *et al*. (2009) Multilocus sequence typing as a tool for studying the molecular epidemiology and population structure of *Brachyspira hyodysenteriae. Vet. Microbiol.* **138**, 330–338. doi:10.1016/j.vetmic.2009.03.025
2. Osorio, J., *et al*. (2012) Dissemination of clonal groups of *Brachyspira hyodysenteriae* amongst pig farms in Spain, and their relationships to isolates from other countries. *PLoS ONE* **7**, e39082. doi:10.1371/journal.pone.0039082
3. Trott, D.J., *et al*. (1998) Population genetic analysis of *Serpulina pilosicoli* and its molecular epidemiology in villages in the Eastern Highlands of Papua New Guinea. *Int. J. Syst. Bacteriol.* **48**, 659–668. doi:10.1099/00207713-48-3-659
4. Bellgard, M.I., *et al*. (2009) Genome sequence of the pathogenic intestinal spirochete *Brachyspira hyodysenteriae* reveals adaptations to its lifestyle in the porcine large intestine. *PLoS ONE* **4**, e4641. doi:10.1371/journal.pone.0004641

5. Mappley, L.J., *et al*. (2012) Comparative genomics of *Brachyspira pilosicoli* strains: extensive genome rearrangements and reductions, and correlation of genetic compliment with phenotypic diversity. *BMC Genomics* **13**, 454. doi:10.1186/1471-2164-13-454

6. Håfström, T., *et al*. (2011) Complete genome sequence of *Brachyspira intermedia* reveals unique genomic features in *Brachyspira* species and phage-mediated horizontal gene transfer. *BMC Genomics* **12**, 395. doi:10.1186/1471-2164-12-395

7. Matson, E.G., *et al*. (2005) Identification of genes of VSH-1, a prophage-like gene transfer agent of *Brachyspira hyodysenteriae*. *J. Bacteriol.* **187**, 5885–5892. doi:10.1128/JB.187.17.5885-5892.2005

8. Motro, Y., *et al*. (2009) Identification of genes associated with prophage-like gene transfer agents in the pathogenic intestinal spirochaetes *Brachyspira hyodysenteriae, Brachyspira pilosicoli* and *Brachyspira intermedia*. *Vet. Microbiol.* **134**, 340–345. doi:10.1016/j.vetmic.2008.09.051

9. Atyeo, R.F., *et al*. (1999) Analysis of *Serpulina hyodysenteriae* strain variation and its molecular epidemiology using pulsed-field gel electrophoresis. *Epidemiol. Infect.* **123**, 133–138. doi:10.1017/S0950268899002691

10. Hidalgo, A., *et al*. (2010) Multiple-locus variable-number tandem-repeat analysis of the swine dysentery pathogen, *Brachyspira hyodysenteriae*. *J. Clin. Microbiol.* **48**, 2859–2865. doi:10.1128/JCM.00348-10

11. Hansen, C.F., *et al*. (2010) Diets containing inulin but not lupins help to prevent swine dysentery in experimentally challenged pigs. *J. Anim. Sci.* **88**, 3327–3336. doi:10.2527/jas.2009-2719

12. Nakamura, S., *et al*. (2006) Improvement in motion efficiency of the spirochete *Brachyspira pilosicoli* in viscous environments. *Biophys. J.* **90**, 3019–3026. doi:10.1529/biophysj.105.074336

13. Naresh, R., *et al*. (2010) Attraction of *Brachyspira pilosicoli* to mucin. *Microbiology* **156**, 191–197. doi:10.1099/mic.0.030262-0

14. Wanchanthuek, P., *et al*. (2010) The complete genome sequence of the pathogenic intestinal spirochete *Brachyspira pilosicoli* and comparison with other *Brachyspira* genomes. *PLoS ONE* **5**, e11455. doi:10.1371/journal.pone.0011455

15. Råsbäck, T., *et al*. (2007) A novel enteropathogenic, strongly haemolytic spirochaete isolated from pig and mallard, provisionally designated '*Brachyspira suanatina*' sp. nov. *Environ. Microbiol.* **9**, 983–991. doi:10.1111/j.1462-2920.2006.01220.x

16. Chander, Y., *et al*. (2012) Phenotypic and molecular characterization of a novel strongly hemolytic *Brachyspira* species, provisionally designated "*Brachyspira hampsonii*". *J. Vet. Diagn. Invest.* **24**, 903–910.

17. Naresh, R., *et al*. (2009) The intestinal spirochete *Brachyspira pilosicoli* attaches to cultured Caco-2 cells and induces pathological changes. *PLoS ONE* **4**, e8352. doi:10.1371/journal.pone.0008352

18. La, T., *et al*. (2011) Evidence that the 36kb plasmid of *Brachyspira hyodysenteriae* contributes to virulence. *Vet. Microbiol.* **153**, 150–155. doi:10.1016/j.vetmic.2011.02.053

19. La, T., *et al*. (2004) Protection of pigs from swine dysentery by vaccination with recombinant BmpB, a 29.7 kDa outer-membrane lipoprotein of *Brachyspira hyodysenteriae*. *Vet. Microbiol.* **102**, 97–109. doi:10.1016/j.vetmic.2004.06.004

20. Song, Y., *et al*. (2009) A reverse vaccinology approach to swine dysentery vaccine development. *Vet. Microbiol.* **137**, 111–119. doi:10.1016/j.vetmic.2008.12.018

21. Mappley, L.J., *et al*. (2013) Evidence for systemic spread of the potentially zoonotic intestinal spirochaete *Brachyspira pilosicoli* in experimentally challenged laying chickens. *J. Med. Microbiol.* **62**, 297–302.

Biography

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Serine proteases and ovine footrot

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Footrot is a disease that is of importance to the wool and sheep meat industries. The principle causative agent of ovine footrot is the anaerobic bacterium, *Dichelobacter nodosus*, virulent isolates of which secrete three closely related subtilisin-like proteases, AprV2, AprV5 and BprV[1]. By constructing isogenic mutants and carrying out virulence tests in sheep it was shown that AprV2 is a major virulence factor of *D. nodosus*[2]. Structural analysis of AprV2 has revealed that it contains several novel loops, one of which appears to act as an exosite that may modulate substrate accessibility[2]. Both elastase activity and protease thermostability have been used for the differential diagnosis of *D. nodosus* isolates. Analysis of the protease mutants has shown that AprV2 is the thermostable protease and also is responsible for the

Figure 1. Gross pathology of a sheep infected with ovine footrot. Separation of the horn of the hoof (A) and lameness (B) are shown.

elastase activity of *D. nodosus*, while AprV5 is the major extracellular protease[2]. In addition, AprV5 is required for its own maturation and for the optimal cleavage of AprV2 and BprV to their mature active forms[3].

The severity of ovine footrot is a continuum that ranges from benign footrot, which presents as an interdigital dermatitis, to virulent footrot, which involves severe underrunning of the horn of the hoof and the separation of the hoof from the underlying tissue (Fig. 1A), leading to lameness (Fig. 1B) and loss of body weight[4–6]. Consequently the disease results in significant losses to the sheep industry due to a reduction in meat and wool production and the cost of control and treatment programs[7,8].

Clinical disease is dependent upon the virulence properties of the causative *D. nodosus* isolate and the presence of warm and wet climatic conditions. Type IV fimbriae and extracellular serine proteases were shown to be major virulence factors following the development of methods for the genetic manipulation of *D. nodosus*[2,9]. Type IV fimbriae also are required for optimal serine protease secretion[9–11].

Virulent strains of *D. nodosus* secrete two acidic proteases, AprV2 and AprV5, and the basic protease BprV, which putatively cause tissue damage during a footrot infection[2,12]. The equivalent proteases in benign strains are known as AprB2, AprB5 and BprB[13,14]. Phenotypic characterisation of the extracellular proteases, including analysis of their elastase activity and protease thermostability, has been traditionally used for the differentiation of benign and virulent strains of *D. nodosus*[15,16], with virulent isolates often producing elastase positive, thermostable proteases and benign strains generally having an elastase negative, more thermolabile protease phenotype. Note that these phenotypes do not absolutely correlate with virulence.

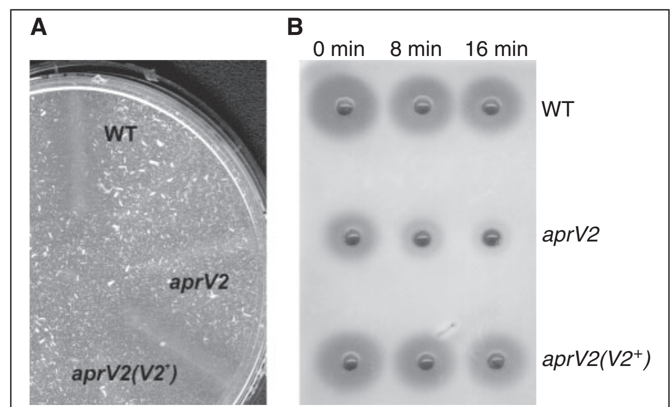

Figure 2. Elastase and protease thermostability. The wild-type strain VCS1703A (WT), its isogenic *aprV2* mutant (*aprV2*) and the complemented derivative (*aprV2*(*V2*$^+$)) were analysed for their elastase activity by plating onto culture medium containing insoluble elastin. Elastase activity is shown by a zone of clearing around the culture streak (A). Protease thermostability was determined by a gelatin-gel test[16], whereby culture supernatants of each strain were tested for protease activity after incubation at 65°C for 0 min, 8 min and 16 min, as shown (B).

These proteases are synthesised as precursors with an N-terminal pre-pro-region, a serine protease domain and a C-terminal extension. The amino acid sequences of the catalytic protease domains are highly conserved (~65% identity)[17–19], but the sequences of the C-terminal extensions are less conserved, showing only approximately 35% similarity. The active proteases are produced by cleavage of the pre-pro region and the C-terminal extension[17,18,20]. Sequence analysis of the C-terminal extensions revealed that they contain a P-domain, which is typically associated with eukaryotic pro-protein convertases that belong to the subtilisin-like protease superfamily[6,21].

To determine the role of each of the proteases in virulence, mutants of each of the three protease genes were constructed by allelic exchange in the virulent strain VCS1703A[2]. Quantitative protease

assays of culture supernatants, using azocasein as the substrate, revealed that AprV5 made the major contribution to overall protease activity; followed by AprV2, with BprV only making a minor contribution[2]. Double mutants had very little protease activity, suggesting that the proteases may act synergistically, or that one or more may be involved in the activation of the other proteases[2]. The mechanism of processing of the extracellular proteases was further examined by zymogram analysis and Western blotting using AprV5-, AprV2- and BprV-specific antisera[3]. The results indicated that AprV5 is responsible for its own maturation and for the optimal processing of both AprV2 and BprV. By constructing a series of C-terminal truncated *aprV5* mutants in *D. nodosus*, it also was shown that the C-terminal extension of AprV5 is required for efficient processing of all three enzymes, presumably because it is required for the optimal self-processing of AprV5. In the absence of this domain, protease processing is delayed[3]. Moreover, it was shown that cleavage of the pro-domain and the C-terminal extensions of the AprV2 and BprV precursors occurs after secretion.

Elastase assays revealed that AprV2 is responsible for the elastase activity found in most virulent isolates of *D. nodosus* (Fig. 2A)[2]. AprV2 is also the major thermostable protease (Fig. 2B). Complementation of the *aprV2* mutant with the benign protease gene *aprB2* restored the overall protease activity to that of the wild type, but not the elastase activity or thermostability; therefore this strain had the phenotype of a benign strain, despite possessing two functional virulent protease genes, *aprV5* and *bprV*.

The virulent wild-type strain, each of the protease mutants, and their corresponding complemented derivatives, were examined in a sheep virulence trial to determine the role of the proteases in disease[2]. The *aprV2* mutant was avirulent and complementation of the mutant with the wild-type *aprV2* gene restored full virulence. Surprisingly, complementation with *aprB2* (the equivalent protease from a wild-type benign strain) also restored full virulence, which indicates that elastase activity is not required for virulence. The *aprV5* and the *bprV* mutants were also avirulent, but unexpectedly their complemented strains were not virulent, possibly due to unstable protease expression in the complemented strains. Therefore, although we cannot conclude that AprV5 and BprV are essential for virulence, it is likely that they do play a role in the disease process.

It has been known for some time that the mature AprV2 and AprB2 proteases differ by a single amino acid, with Tyr92 of AprV2 substituted by an Arg residue in AprB2[18]. To better understand how this single amino acid change could contribute to the substrate specificity of these proteases the crystal structures of both proteases were determined[2]. The structures were closely related and were similar to other subtilisin-like proteases, although they each had several major insertions (I1 – I4) in the loops surrounding the active site cleft. The largest of these insertions (I2) is tethered by a disulphide bond, and the single amino acid change (Y92R) between AprV2 and AprB2 is located at the end of this loop. Site-directed mutagenesis revealed that Tyr92 does not contribute to catalysis at the active site, but that the I2 loop acts as an exosite and mediates the formation of a stable enzyme-substrate interaction. The tethering of the loop by the disulphide bond appears to be important for this function[2]. Finally, analysis of the crystal structures of BprV and BprB reveals that differences in the substrate specificity of these enzymes reflects amino acid changes in the S1 pocket of these enzymes and that subtle changes in the *D. nodosus* proteases may significantly influence tissue destruction[22].

Conclusions

For many years extracellular serine proteases had been considered as putative virulence factors of *D. nodosus*; however, their importance in the pathogenesis of disease has only recently been established. There is now clear evidence that AprV2 is the major thermostable protease and elastase in virulent strains of *D. nodosus* and is essential for virulence. In addition, it has been shown that AprV5 is required for its self-maturation and that it facilitates the activation of AprV2 and BprV, a process that requires the C-terminal extension of AprV5. The fact that a strain in which an *aprV2* mutation was complemented with the *aprB2* gene was benign by standard laboratory diagnostic tests, but caused virulent disease in sheep, indicates that elastase activity and thermostability are not direct indicators of the virulence of a strain. This finding is in keeping with the difficult task of precisely defining a virulent isolate of *D. nodosus,* an issue that has been noted for many years in this field. The presence of other virulence factors, such as the other virulent proteases and type IV fimbriae-mediated twitching motility, also contributes to virulence. Virulence in *D. nodosus* clearly is multifactorial and we suspect that the pathogenesis of disease is much more complex than currently envisaged.

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References

1. Kennan, R.M., *et al*. (2011) The pathogenesis of ovine footrot. *Vet. Microbiol.* **153**, 59–66. doi:10.1016/j.vetmic.2011.04.005
2. Kennan, R.M., *et al*. (2010) The subtilisin-like protease AprV2 is required for virulence and uses a novel disulphide-tethered exosite to bind substrates. *PLoS Pathog.* **6**, e1001210. doi:10.1371/journal.ppat.1001210
3. Han, X., *et al*. (2012) The AprV5 subtilase is required for the optimal processing of all three extracellular serine proteases from *Dichelobacter nodosus*. *PLoS ONE* **7**, e47932. doi:10.1371/journal.pone.0047932

4. Egerton, J.R., *et al*. (1969) The aetiology and pathogenesis of ovine footrot. I.A histological study of the bacterial invasion. *J. Comp. Pathol.* **79**, 207–216. doi:10.1016/0021-9975(69)90007-3
5. Stewart, D.J., *et al*. (1984) Differences between strains of *Bacteroides nodosus* in their effects on the severity of footrot, bodyweight and wool growth on Merino sheep. *Aust. Vet. J.* **61**, 348–352. doi:10.1111/j.1751-0813.1984.tb07153.x
6. Siezen, R.J. and Leunissen, J.A. (1997) Subtilases: the superfamily of subtilisin-like serine proteases. *Protein Sci.* **6**, 501–523. doi:10.1002/pro.5560060301
7. Egerton, J.R. (1986) Control and eradication of ovine footrot. In *Footrot in Ruminants. Proceedings of a Workshop, Melbourne 1985* (Stewart, D.J. *et al.*, eds), pp. 35–42, CSIRO Division of Animal Health and Australian Wool Corporation.
8. Green, L.E. and George, T.R. (2008) Assessment of current knowledge of footrot in sheep with particular reference to *Dichelobacter nodosus* and implications for elimination or control strategies for sheep in Great Britain. *Vet. J.* **175**, 173–180. doi:10.1016/j.tvjl.2007.01.014
9. Kennan, R.M., *et al*. (2001) The type IV fimbrial subunit gene (*fimA*) of *Dichelobacter nodosus* is essential for virulence, protease secretion, and natural competence. *J. Bacteriol.* **183**, 4451–4458. doi:10.1128/JB.183.15.4451-4458.2001
10. Han, X., *et al*. (2008) Twitching motility is essential for virulence in *Dichelobacter nodosus*. *J. Bacteriol.* **190**, 3323–3335. doi:10.1128/JB.01807-07
11. Han, X., *et al*. (2007) Type IV fimbrial biogenesis is required for protease secretion and natural transformation in *Dichelobacter nodosus*. *J. Bacteriol.* **189**, 5022–5033. doi:10.1128/JB.00138-07
12. Thomas, J.H. (1964) The pathogenesis of footrot in sheep with reference to proteases of *Fusiformis nodosus*. *Aust. J. Agric. Res.* **15**, 1001–1016. doi:10.1071/AR9641001
13. Kortt, A.A., *et al*. (1993) Amino acid sequence of extracellular acidic protease V5 of *Dichelobacter nodosus*, the causative organism of ovine footrot. *Biochem. Mol. Biol. Int.* **29**, 989–998.
14. Kortt, A.A., *et al*. (1994) Characterization of a basic serine protease (pI ~ 9.5) secreted by virulent strains of *Dichelobacter nodosus* and identification of a distinct, but closely related, proteinase secreted by benign strains. *Biochem. J.* **299**, 521–525.
15. Stewart, D.J. (1979) The role of elastase in the differentiation of *Bacteroides nodosus* infections in sheep and cattle. *Res. Vet. Sci.* **27**, 99–105.
16. Palmer, M.A. (1993) A gelatin test to detect activity and stability of proteases produced by *Dichelobacter nodosus*. *Vet. Microbiol.* **36**, 113–122. doi:10.1016/0378-1135(93)90133-R
17. Riffkin, M.C., *et al*. (1993) Cloning, sequence and expression of the gene (*aprV5*) encoding extracellular serine acidic protease V5 from *Dichelobacter nodosus*. *Gene* **137**, 259–264. doi:10.1016/0378-1119(93)90017-W
18. Riffkin, M.C., *et al*. (1995) A single amino-acid change between the antigenically different extracellular serine proteases V2 and B2 from *Dichelobacter nodosus*. *Gene* **167**, 279–283. doi:10.1016/0378-1119(95)00664-8
19. Lilley, G.G., *et al*. (1995) Nucleotide and deduced protein sequence of the extracellular, serine basic protease gene *bprB* from *Dichelobacter nodosus* strain 305: comparison with the basic protease gene *bprV* from virulent strain 198. *Biochem. Mol. Biol. Int.* **36**, 101–111.
20. Lilley, G.G., *et al*. (1992) Amino acid and DNA sequences of an extracellular basic protease of *Dichelobacter nodosus* show that it is a member of the subtilisin family of proteases. *Eur. J. Biochem.* **210**, 13–21. doi:10.1111/j.1432-1033.1992.tb17385.x
21. Shinde, U. and Thomas, G. (2011) Insights from bacterial subtilases into the mechanisms of intramolecular chaperone-mediated activation of furin. *Methods Mol. Biol.* **768**, 59–106. doi:10.1007/978-1-61779-204-5_4
22. Wong, W., *et al*. (2011) S1 pocket of a bacterially derived subtilisin-like protease underpins effective tissue destruction. *J. Biol. Chem.* **286**, 42 180–42 187. doi:10.1074/jbc.M111.298711

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Dr Ruth Kennan is a Research Fellow within the ARC Centre of Excellence in the Department of Microbiology at Monash University. Her research interests are in the pathogenesis and virulence of *Dichelobacter nodosus,* the causative agent of ovine footrot. She is responsible for the development of methods for the genetic manipulation of *D. nodosus*. Current work involves looking at regulation of virulence factors and looking for genomic differences between benign and virulent strains.

Prof Julian Rood is a Chief Investigator of the ARC Centre of Excellence and Deputy Head of the Department of Microbiology at Monash University. His research group has worked for many years on the genetics and pathogenesis of anaerobic bacteria, particularly the pathogenic clostridia and *Dichelobacter nodosus*. He is a Past President and Fellow of the Australian Society for Microbiology and a Fellow of the American Academy of Microbiology.

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Bovine ephemeral fever: cyclic resurgence of a climate-sensitive vector-borne disease

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Bovine ephemeral fever is one of Australia's most important viral diseases of cattle. It is caused by a rhabdovirus that is transmitted by haematophagous insects, most likely mosquitoes, producing seasonal epizootics that can have serious impacts on beef and dairy production. Since 2008, extreme summer rainfall and extensive flooding have provided ideal conditions for the emergence of large mosquito populations, accompanied by successive extensive epizootics of bovine ephemeral fever. Climate change predictions of the increasing intensity and frequency of alternating droughts and severe La Niña events in Australia suggest there is a need to explore new intervention strategies to reduce or eliminate the future impacts of this costly disease.

Bovine ephemeral fever (BEF) is a debilitating, and sometimes fatal, viral disease of cattle and water buffalo. Also called three-day sickness (and various other local names), it is enzootic in tropical and sub-tropical regions of Africa, Asia and Australia, surging seasonally into temperate zones in sometimes extensive epizootics. It does not occur in most of Europe, the Pacific Islands or the Americas, where it is considered a significant exotic disease threat. The causative agent, bovine ephemeral fever virus (BEFV) is an arthropod-borne rhabdovirus (genus *Ephemerovirus*, family *Rhabdoviridae*)[1]. Although the disease is non-contagious and the epizootiology is consistent with insect-borne transmission, the vectors of BEF are not well defined. The virus has been isolated from both mosquitoes and biting midges (*Culicoides* spp) in Australia and Africa, but various factors suggest that mosquitoes are the principal vectors. Only one BEFV serotype exists worldwide and infection apparently leads to life-long immunity[2].

The clinical signs of BEF can be severe but are usually transient and can include anorexia, rumenal stasis, nasal and ocular discharge, excessive salivation, stiffness, joint swelling, rear limb paralysis and a biphasic fever. Typically, during extensive epizootics morbidity rates are very high (up to 80% in some herds) and, although mortality rates are usually quite low (less than 1%), deaths are more likely to occur in older, heavier, more valuable animals[3]. Due to the transient nature of the disease and low mortality rates, the economic impact of BEF is often underappreciated. Disease results in decreased milk production in dairy herds, delayed oestrus and mid-term abortions in cows, temporary infertility in bulls, and loss of condition in beef herds. The economic loss during a severe epizootic season in Australia can be as high as $100–200 million.

In recent years, major BEF epizootics have occurred in Taiwan and China, the Middle-East, and Australia, in some cases with unusually high mortality rates. Mortalities due to disease and culling of affected animals were reported to be as high as 11.3% and 21.9% in Taiwan in 1996 and 1999, respectively[4]. A mortality rate of 8.6% was reported in Jordan in 1999[5] and a severe epizootic with high mortalities (12% of clinical cases) was reported in NSW in 2001. In Henan Province in China, mortality rates have been reported to have increased significantly since 2000, estimated at 18% of cases in 2004 and 2005, and 5% in 2011[6]. It is not clear at this stage if the increased severity of the disease is associated with changes in virulence of the virus or environmental factors that have increased host susceptibility to infection.

In Australia, BEF has been known since 1936 when a sweeping epizootic surged out of the Top End of the Northern Territory (Fig. 1) spreading westward into the Kimberleys and eastward through NSW and Queensland, reaching Victoria in the late summer of 1937[3]. The prevalence of infection was very high, approaching 100% in northern areas. Similar wave-like epizootics occurred in 1955–56 and 1967–68, and then several occurred in succession during an intense La Niña period that persisted until 1976. The epizootiology of the disease in Australia then changed dramatically, with the virus becoming enzootic throughout a vast area of eastern Australia as far south as the Hunter Valley in NSW. Although the disease remained perennially active in the Top End, in Queensland and NSW the pattern was characterised by annual sporadic local outbreaks with occasional extensive epizootics during periods of heavy rainfall[3]. Surveillance data from sentinel herds indicated annual seroconversions in the eastern states and Western Australia, with very low levels of transmission during extended periods of drought, particularly during winter and spring.

In the summer of 2007–2008, after a period of prolonged drought, a return to La Niña conditions with record rains and extensive flooding

Figure 1. Top End of the Northern Territory, Australia.

throughout much of eastern Australia caused a surge in mosquito populations and another extensive BEF epizootic. Large numbers of diseased animals were reported throughout the Northern Territory and Queensland with high morbidity and mortality rates, particularly in the severely flooded Belyando District in Central Queensland. In New South Wales, a widespread epizootic commenced near Bourke in the north-west of the state in the first week of January 2008 and progressed rapidly to the Victorian border over a period of 2–3 weeks. As in the epizootic of 1970–71, the southerly sweep of the 2008 epizootic in NSW was initially west of the Great Dividing Range with transmission subsequently occurring on the coast. This appears to have been due to the deposition of insects carried from Queensland in an intense low pressure system that moved through the region at that time[7]. BEF remained very active in Queensland during the summer and autumn of 2009 and then, in early 2010, widespread flooding across much of eastern Australia resulted in another extensive epizootic, with the virus entering northern Victoria for the first time since 1996.

With the imminent return to El Niño conditions to Australia in coming years, we will undoubtedly see a decline in the BEFV transmission throughout much of eastern Australia and, as in the past, the disease will again assume less importance to farmers. However, as rainfall declines and leads inevitably to drought, and as vector activity declines and leads inevitably to low herd immunity, the large susceptible cattle population will inevitably host future extensive epizootics. An effective BEF vaccine is available in Australia but its adoption rate in northern beef herds is low primarily because of the need to muster for multiple vaccinations to get effective protection and its use in dairy herds declines as epizootics subside. Nevertheless, the availability of the vaccine presents the opportunity for more effective intervention and the seasonal characteristics of the disease raise the interesting question of the potential for future eradication.

Phylogenetic analyses conducted using the complete ectodomain region of the BEFV G gene (encoding the surface transmembrane glycoprotein) indicate that the Australian clade is a single lineage, which is evolving at a relatively constant rate, and that all Australian isolates are derived from a single ancestral virus that was introduced approximately 60 years ago. Although West Australian isolates have not been analysed to date, the absence of regional sub-lineages amongst 100 or more isolates analysed from Queensland, NSW and the NT indicates that the virus is continually extinguished in the eastern states due to transmission failure, and replenished regularly by strains from the tropical endemic focus in the Top End. Could we therefore, through targeted vaccination, halt completely the low level of transmission that occurs during winter months, particularly in periods of severe drought? As infection leads to lifelong immunity and a durable vaccine is available, this appears to be feasible in the eastern states where winter seroconversions are sometimes not detected. However, the Top End presents a more significant challenge as beef herds are large and remote and feral water buffalo are likely to be involved in the epizootiology. The molecular epizootiology clearly shows that eradication in eastern states would be temporary if there remained an endemic focus in the North. Nevertheless, it is clear that the impacts of BEF in Australia are substantial during periods of cyclic resurgence such as we have seen recently, and more effective intervention strategies are badly needed.

References

1. Dietzgen, R.G., *et al*. (2011) *Rhabdoviridae*. In *Virus Taxonomy, 9th Report of the ICTV* (King, A.M.Q. *et al.*, eds), pp. 654–681, Elsevier.
2. Walker, P.J., *et al*. (2012) Ephemeroviruses: arthropod-borne rhabdoviruses of ruminants, with large and complex genomes. In *Rhabdoviruses: Molecular Taxonomy, Evolution, Genomics, Ecology, Cytopathology and Control* (Dietzgen, R.G., and Kuzmin, I.V., eds), pp. 59–88, Horizon Scientific Press.
3. Walker, P.J. (2005) Bovine ephemeral fever in Australia and the world. *Curr. Top. Microbiol. Immunol.* **292**, 57–80. doi:10.1007/3-540-27485-5_4
4. Hsieh, Y.-C., *et al*. (2005) Bovine ephemeral fever in Taiwan (2001–2002). *J. Vet. Med. Sci.* **67**, 411–416. doi:10.1292/jvms.67.411
5. Yeruham, I., *et al*. (2002) Epidemiological investigations of outbreaks of bovine ephemeral fever in Israel. *Vet. Rec.* **151**, 117–121. doi:10.1136/vr.151.4.117
6. Zheng, F.Y. and Qui, C.Q. (2012) Phylogenetic relationships of the glycoprotein gene of bovine ephemeral fever virus isolated from mainland China, Taiwan, Japan, Turkey, Israel and Australia. *Virol. J.* **9**, 268. doi:10.1186/1743-422X-9-268
7. Finlaison, D.S., *et al*. (2010) An epizootic of bovine ephemeral fever in New South Wales in 2008 associated with long-distance dispersal of vectors. *Aust. Vet. J.* **88**, 301–306. doi:10.1111/j.1751-0813.2010.00596.x

Biography

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Multi-drug-resistant Gram-negative bacteria

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The increasing prevalence of antibiotic-resistant Gram-negative bacteria is a serious concern not only for humans but also companion animals[1–3]. Recent clinical attention has focused on the increasing frequency of Gram-negative pathogens responsible for hospital-acquired infections. In this group, extended-spectrum β-lactamase (ESBL) and carbapenemase producing Enterobacteriaceae, in particular *E. coli* and *K. pneumoniae,* as well as carbapenemase producing *A. baumannii*, have caused major challenges in the past decade. These three groups of organisms will be the focus of this article.

β-lactams are the most widely utilised antimicrobials worldwide for the treatment of infections in humans and animals. β-lactamase production is the most common mechanism of antibiotic resistance, particularly in Gram-negative bacilli. There are four different groups based on the Ambler molecular classification, which categorises the enzyme according to their amino acid sequence, primarily on their active site. Classes A, C and D are serine β-lactamases and Class B are metallo-β-lactamases which require a bivalent metal ion for activity, usually Zn^{2+} (Table 1)[4].

ESBL and CMY-2-like producing *Enterobacteriaceae*

Two types of cephalosporinases commonly described in human and animal pathogens are ESBLs and CMY-2 like. CMY-2 type of resistance was commonly described in *Salmonella* and *E. coli* isolated from food-producing animals[5,6]. CMY-2 producing *E. coli* is also relatively common in humans and nearly as common as ESBL producing *E. coli*[7]. Nowadays, CTX-M type ESBLs are the most commonly reported β-lactamases both in human and animals. Until the year 2000, TEM and SHV type ESBLs were of great concern and mostly in *E. cloacae* and *K. pneumoniae*. However, since mid-2000s, there has been a dramatic shift into the predominance of CTX-M type ESBLs[8]. In dogs and cats, there have been reports of infections caused by CTX-M type ESBL producing *E. coli* associated with human infections[9,10]. In the USA, CTX-M type ESBL, mostly CTX-M-15 occurred at the rate of 7.3% amongst *E. coli* causing urinary tract infections in dogs[9] and caused serious infections, such as liver and bile infection in dogs[10]. CTX-M producing *E. coli* was quite common in pig farms in China, with the prevalence of 10.7%[11]. The presence of this ESBL in farm animal causes a significant threat to public health.

E. coli ST131 clone

E. coli is a leading cause of urinary tract infections in humans and dogs. *E. coli* can be divided into four phylogenetic groups: A, B1, B2 and D. The virulent extra-intestinal pathogens are usually found in classes B2 and D[12]. In the last decade, *E. coli* sequence type (ST) 131 has emerged as a pandemic uropathogenic *E. coli* causing community and hospital-acquired infections especially urinary tract infections[13,14]. This clone potentially can harbour a variety of β-lactamase genes; however, it is most strongly associated with CTX-M-15 ESBL. In addition, the pandemic clone *E. coli* ST131 also belonged to the phylogenetic group B2, the highly virulent group of ExPEC. The combination of virulence and antimicrobial resistance may give *E. coli* ST131 a fitness advantage over other *E. coli* strains,

Table 1. Classes of β-lactamase in *Enterobacteriaceae* and *Acinetobacter* spp.

Ambler classification	Description or characteristics	Examples of enzymes	Bacterial strains
Class A (serine β-lactamase)	Cephalosporinases (ESBLs) Usually clavulanic acid susceptible, except for KPC	TEM, SHV, CTX-M, **KPC**, VEB	*Enterobacteriaceae, Pseudomonas* spp.
Class B (metallo-β-lactamase or MBL)	Contain metal ion (Zn) Carbapenemases Not inhibited by clavulanic acid Inhibited by aztreonam	**IMP, VIM, NDM**	*Enterobacteriaceae, Acinetobacter* spp., *Pseudomonas* spp.
Class C (AmpC β-lactamase – serine β-lactamase)	Resistant to clavulanic acid Intrinsic in certain species of Gram-negative	CMY, DHA	*Enterobacteriaceae*
Class D (serine β-lactamase)	Oxacillinases Susceptible to clavulanic acid Carbapenemase	**OXA**	*Enterobacteriaceae* (OXA-48 like), *Acinetobacter* spp.

Note: Enzymes in bold are carbapenemases.

which highlights the widespread and successful dissemination of *E. coli* ST131.

E. coli ST131 is also represented among resistant isolates in companion and farm animals. A European study determined the presence of *E. coli* ST131 which mostly produced CTX-M-15, comprising 5.6% of ESBL-producing *E. coli* isolates recovered from companion animals[15]. In Australia, *E. coli* ST131 accounted for 7.2% of isolates from companion animals[16]. Transmission between dogs and cats sharing the same clone, *E. coli* ST131, has been described previously[17]. Dogs in particular have been identified as a possible reservoir not only for ST131, but also for extra-intestinal pathogenic *E. coli* (ExPEC) in general for the transmission to other pets and humans[18]. *E. coli* ST131 has also been reported from poultry and pig farms in Spain[19]. In this instance, both companion and farm animals can be the reservoirs for *E. coli* ST131 and other cephalosporin-resistant *E. coli*. Studies on retail meat for human consumption have found *E. coli* indistinguishable from human *E. coli* causing urinary tract infections (UTIs) and *E. coli* ST131 was isolated from chicken[20]. Further studies are urgently needed to investigate the pathways of transmission between humans and animals to determine whether isolates found in retail meat and livestock pose an imminent threat to humans.

Carbapenem-resistant *Enterobacteriaceae* (CRE)

Various *Enterobacteriaceae* confer antibiotic resistance by producing enzymes such as carbapenemases. The carbapenemase modifies the carbapenems through the hydrolysis of the antibiotic and has been defined into different classes using the Ambler classification system. The resistance in *Enterobacteriaceae* is found within Ambler class A (KPC – *K. pneumoniae* Carbapenemase), class B (metallo-β-lactamases) and class D (OXA – oxacillinases) (Table 1)[21]. KPC-type β-lactamases, are the most frequent cause of carbapenem resistance in the United States and has spread rapidly to Europe[22,23]. The most recent class B enzyme identified is the New Delhi Metallo–β-lactamase (NDM-1) in 2009[24]. These enzymes are frequently found in *K. pneumoniae* and *E. coli.* The majority of NDM-producing isolates are found on the Indian subcontinent[25].

The recent emergence of CRE represents a significant threat and has also drawn substantial media attention, especially through the spread of the NDM-1[26]. The NDM-1 gene was first described from a Swedish patient who travelled to India in 2008, but this gene has now spread worldwide[25]. NDM-1 can be found in numerous species of *Enterobacteriaceae*, including *E. coli* and various species of *Acinetobacter* spp.[27]. NDM gene is able to spread rapidly by residing in self-transferrable plasmids. NDM-1 producers have been isolated from water and other environmental samples in India[28]. A total of 88 NDM-1 cases were reported in the United Kingdom (up to March 2011[29]); 13 NDM-1 cases were reported in the USA[30]. Eight cases have been reported in Australia. Most cases identified outside India and Pakistan, including those in Australia and New Zealand, were related to travel and/or medical treatment in India or Pakistan[24,31]. There have been several cases due to local acquisition of the NDM producers or without travel history in Europe and Asia[32,33]. The rapid spread of NDM has moved outside the hospital. Studies in China found NDM-1 producing *Acinetobacter* spp. from hospital waste[34] and food of animal origin[35]. The worldwide spread of NDM producers in hospitals and the environment are clearly a real threat.

Carbapenem-resistant *Acinetobacter baumannii*

A. baumannii has gained recognition as a major nosocomial pathogen in recent years[36] predominantly affecting immunocompromised or critically ill patients. It causes a wide range of infections including pneumonia, bacteraemia and infections of the skin, bone, urinary tract and central nervous system[36]. The remarkable ability of *A. baumannii* to up-regulate or acquire antibiotic resistance determinants makes the bacterium a significant nosocomial pathogen. Resistance to carbapenems has been reported worldwide, including Australia[37–39]. Many genotypes conferring such resistance have been found to belong to European clones (EU) I, II or III[37]. Although several molecular mechanisms were responsible for conferring carbapenem resistance, the most common is the production of class D β-lactamases, OXA-type carbapenemases. Class B metallo-β-lactamases (such as IMP-, VIM- and SIM-types) are occasionally identified in *A. baumannii* isolates which have acquired these resistant determinants via class 1 integrons (Table 1)[40]. Alteration of penicillin-binding proteins and loss of outer membrane proteins[41], efflux pump mechanisms and other β-lactamases are also found in *A. baumannii*[42].

The OXA-type carbapenemases identified in *A. baumannii* include both (i) acquired types (OXA-23-, OXA-24- and OXA-58-like) where their gene clusters have been found either in the chromosome or plasmid and (ii) naturally occurring chromosomal OXA-51-like[43]. Multiple studies regarding genomic sequences surrounding these genes revealed the pivotal role of insertion sequence (IS) elements. The presence of IS*Aba1* upstream of $bla_{\text{OXA-23}}$ as well as intrinsic chromosomal $bla_{\text{OXA-51-like}}$ provide promoter sequences consequently leading to the over-expression of these downstream genes[40,44]. IS*Aba1* and other IS elements, such as IS*Aba2*, IS*Aba3* and IS*8* were shown to enhance the expression of $bla_{\text{OXA-58}}$[45]. These IS elements play significant roles in the expression of various OXA-genes in *A. baumannii. A. baumannii* can also be isolated from faeces of slaughtered pigs and cattle[46]. These strains from animals possessed $bla_{\text{OXA-51-like}}$, however, the resistance islands could not be found and none of the isolates from animals belonged to the European clones I, II and III as the common clones of human *A. baumannii* strains[46].

The rapid spread of multi-drug-resistant bacteria Gram-negative bacteria, especially in nosocomial pathogens; in the past decade underline the importance of infection control precautions in both human and veterinary hospitals. The possibility that farm animals may be the reservoir of resistance mechanisms and the current evidence of spread of the most recent and notorious carbapenem resistance genes demonstrate the need for increased surveillance of antibiotic usage in veterinary and farm animals as well as in humans.

References

1. Sanchez, S., *et al*. (2002) Characterization of multidrug-resistant *Escherichia coli* isolates associated with nosocomial infections in dogs. *J. Clin. Microbiol.* **40**, 3586–3595. doi:10.1128/JCM.40.10.3586-3595.2002
2. Sidjabat, H.E., *et al*. (2006) Identification of $bla_{\text{CMY-7}}$ and associated plasmid-mediated resistance genes in multidrug-resistant *Escherichia coli* isolated from dogs at a veterinary teaching hospital in Australia. *J. Antimicrob. Chemother.* **57**, 840–848. doi:10.1093/jac/dkl057
3. Sidjabat, H.E., *et al*. (2006) Emergence and spread of two distinct clonal groups of multidrug-resistant *Escherichia coli* in a veterinary teaching hospital in Australia. *J. Med. Microbiol.* **55**, 1125–1134. doi:10.1099/jmm.0.46598-0
4. Ambler, R.P. ((1980)) The structure of beta-lactamases. *Phil. Trans. Royal Soc. London. Series B, Biological sciences* **289**, 321–331.
5. Mataseje, L.F., *et al*. (2010) Comparison of CMY-2 plasmids isolated from human, animal, and environmental *Escherichia coli* and *Salmonella* spp. from Canada. *Diagn. Microbiol. Infect. Dis.* **67**, 387–391. doi:10.1016/j.diagmicrobio.2010.02.027
6. Winokur, P.L., *et al*. (2000) Animal and human multidrug-resistant, cephalosporin-resistant salmonella isolates expressing a plasmid-mediated CMY-2 AmpC beta-lactamase. *Antimicrob. Agents Chemother.* **44**, 2777–2783. doi:10.1128/AAC.44.10.2777-2783.2000
7. Sidjabat, H.E., *et al*. (2009) Clinical features and molecular epidemiology of CMY-type beta-lactamase-producing *Escherichia coli. Clin. Infect. Dis.* **48**, 739–744. doi:10.1086/597037
8. Livermore, D.M., *et al*. (2007) CTX-M: changing the face of ESBLs in Europe. *J. Antimicrob. Chemother.* **59**, 165–174. doi:10.1093/jac/dkl483
9. O'Keefe, A., *et al*. (2010) First detection of CTX-M and SHV extended-spectrum beta-lactamases in *Escherichia coli* urinary tract isolates from dogs and cats in the United States. *Antimicrob. Agents Chemother.* **54**, 3489–3492. doi:10.1128/AAC.01701-09
10. Timofte, D., *et al*. (2011) Detection of extended-spectrum-beta-lactamase-positive *Escherichia coli* in bile isolates from two dogs with bacterial cholangiohepatitis. *J. Clin. Microbiol.* **49**, 3411–3414. doi:10.1128/JCM.01045-11
11. Tian, G.B., *et al*. (2009) Detection of CTX-M-15, CTX-M-22, and SHV-2 extended-spectrum beta-lactamases (ESBLs) in *Escherichia coli* fecal-sample isolates from pig farms in China. *Foodborne Pathog. Dis.* **6**, 297–304. doi:10.1089/fpd.2008.0164
12. Johnson, J.R., *et al*. (2006) Phylogenetic relationships among clonal groups of extraintestinal pathogenic *Escherichia coli* as assessed by multi-locus sequence analysis. *Microbes Infect.* **8**, 1702–1713. doi:10.1016/j.micinf.2006.02.007
13. Rogers, B.A., *et al*. (2011) *Escherichia coli* O25b-ST131: a pandemic, multi-resistant, community-associated strain. *J. Antimicrob. Chemother.* **66**, 1–14. doi:10.1093/jac/dkq415
14. Sidjabat, H.E., *et al*. (2010) *Escherichia coli* ST131 producing CTX-M-15 in Australia. *J. Antimicrob. Chemother.* **65**, 1301–1303. doi:10.1093/jac/dkq098
15. Ewers, C., *et al*. (2010) Emergence of human pandemic O25:H4-ST131 CTX-M-15 extended-spectrum-beta-lactamase-producing *Escherichia coli* among companion animals. *J. Antimicrob. Chemother.* **65**, 651–660. doi:10.1093/jac/dkq004
16. Platell, J.L., *et al*. (2010) Clonal group distribution of fluoroquinolone-resistant *Escherichia coli* among humans and companion animals in Australia. *J. Antimicrob. Chemother.* **65**, 1936–1938. doi:10.1093/jac/dkq236
17. Johnson, J.R., *et al*. (2009) Sharing of *Escherichia coli* sequence type ST131 and other multidrug-resistant and urovirulent *E. coli* strains among dogs and cats within a household. *J. Clin. Microbiol.* **47**, 3721–3725. doi:10.1128/JCM.01581-09
18. Johnson, J.R., *et al*. (2009) Virulence genotypes and phylogenetic background of fluoroquinolone-resistant and susceptible *Escherichia coli* urine isolates from dogs with urinary tract infection. *Vet. Microbiol.* **136**, 108–114. doi:10.1016/j.vetmic.2008.10.006
19. Cortes, P., *et al*. (2010) Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. *Appl. Environ. Microbiol.* **76**, 2799–2805. doi:10.1128/AEM.02421-09
20. Vincent, C., *et al*. (2010) Food reservoir for *Escherichia coli* causing urinary tract infections. *Emerg. Infect. Dis.* **16**, 88–95. doi:10.3201/eid1601.091118

21. Nordmann, P., *et al*. (2011) Global spread of Carbapenemase-producing Enterobacteriaceae. *Emerg. Infect. Dis.* **17**, 1791–1798. doi:10.3201/eid1710.110655

22. Cantón, R., *et al*. (2012) Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin. Microbiol. Infect.* **18**, 413–431. doi:10.1111/j.1469-0691.2012.03821.x

23. Kitchel, B., *et al*. (2009) Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. *Antimicrob. Agents Chemother.* **53**, 3365–3370. doi:10.1128/AAC.00126-09

24. Yong, D., *et al*. (2009) Characterization of a new metallo-beta-lactamase gene, $bla_{\mathrm{NDM-1}}$, and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. *Antimicrob. Agents Chemother.* **53**, 5046–5054. doi:10.1128/AAC.00774-09

25. Nordmann, P. (2012) Carbapenem resistance in Enterobacteriaceae: here is the storm! *Trends Mol. Med.* **18**, 263–272. doi:10.1016/j.molmed.2012.03.003

26. Walsh, T.R. and Toleman, M.A. (2012) The emergence of pan-resistant Gram-negative pathogens merits a rapid global political response. *J. Antimicrob. Chemother.* **67**, 1–3. doi:10.1093/jac/dkr378

27. Perry, J.D., *et al*. (2011) Prevalence of faecal carriage of Enterobacteriaceae with NDM-1 carbapenemase at military hospitals in Pakistan, and evaluation of two chromogenic media. *J. Antimicrob. Chemother.* **66**, 2288–2294. doi:10.1093/jac/dkr299

28. Walsh, T.R., *et al*. (2011) Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect. Dis.* **11**, 355–362. doi:10.1016/S1473-3099(11)70059-7

29. UK Health Protection Agency http:www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/CarbapenemResistance/EpidemiologicalData/. accessed 11 November 2012.

30. Centers for Disease Control and Prevention. (2012) Carbapenem-resistant Enterobacteriaceae containing New Delhi metallo-beta-lactamase in two patients – Rhode Island, March 2012. *MMWR Morb. Mortal. Wkly. Rep.* **61**, 446–448.

31. Sidjabat, H., *et al*. (2011) Carbapenem resistance in *Klebsiella pneumoniae* due to the New Delhi Metallo-beta-lactamase. *Clin. Infect. Dis.* **52**, 481–484. doi:10.1093/cid/ciq178

32. Arpin, C., *et al*. (2012) NDM-1-producing *Klebsiella pneumoniae* resistant to colistin in a French community patient without history of foreign travel. *Antimicrob. Agents Chemother.* **56**, 3432–3434. doi:10.1128/AAC.00230-12

33. Rimrang, B., *et al*. (2012) Emergence of NDM-1- and IMP-14a-producing Enterobacteriaceae in Thailand. *J. Antimicrob. Chemother.* **67**, 2626–2630. doi:10.1093/jac/dks267

34. Chen, Y., *et al*. (2011) Emergence of NDM-1-producing *Acinetobacter baumannii* in China. *J. Antimicrob. Chemother.* **66**, 1255–1259. doi:10.1093/jac/dkr082

35. Wang, Y., *et al*. (2012) Identification of New Delhi metallo-beta-lactamase 1 in *Acinetobacter lwoffii* of food animal origin. *PLoS ONE* **7**, e37152. doi:10.1371/journal.pone.0037152

36. Peleg, A.Y., *et al*. (2008) *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin. Microbiol. Rev.* **21**, 538–582. doi:10.1128/CMR.00058-07

37. Higgins, P.G., *et al*. (2010) Global spread of carbapenem-resistant *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* **65**, 233–238. doi:10.1093/jac/dkp428

38. Post, V., *et al*. (2010) Evolution of AbaR-type genomic resistance islands in multiply antibiotic-resistant *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* **65**, 1162–1170. doi:10.1093/jac/dkq095

39. Runnegar, N., *et al*. (2010) Molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* in a single institution over a 10-year period. *J. Clin. Microbiol.* **48**, 4051–4056. doi:10.1128/JCM.01208-10

40. Poirel, L. and Nordmann, P. (2006) Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. *Clin. Microbiol. Infect.* **12**, 826–836. doi:10.1111/j.1469-0691.2006.01456.x

41. Mussi, M.A., *et al*. (2007) CarO, an *Acinetobacter baumannii* outer membrane protein involved in carbapenem resistance, is essential for L-ornithine uptake. *FEBS Lett.* **581**, 5573–5578. doi:10.1016/j.febslet.2007.10.063

42. Vila, J., *et al*. (2007) Porins, efflux pumps and multidrug resistance in *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* **59**, 1210–1215. doi:10.1093/jac/dkl509

43. Turton, J.F., *et al*. (2006) Identification of *Acinetobacter baumannii* by detection of the $bla_{\mathrm{OXA-51}}$-like carbapenemase gene intrinsic to this species. *J. Clin. Microbiol.* **44**, 2974–2976. doi:10.1128/JCM.01021-06

44. Turton, J.F., *et al*. (2006) The role of IS*Aba1* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. *FEMS Microbiol. Lett.* **258**, 72–77. doi:10.1111/j.1574-6968.2006.00195.x

45. Poirel, L. and Nordmann, P. (2006) Genetic structures at the origin of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene $bla_{\mathrm{OXA-58}}$ in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **50**, 1442–1448. doi:10.1128/AAC.50.4.1442-1448.2006

46. Hamouda, A., *et al*. (2011) Epidemiology of *Acinetobacter baumannii* of animal origin. *Int. J. Antimicrob. Agents* **38**, 314–318. doi:10.1016/j.ijantimicag.2011.06.007

Biographies

Dr Hanna Sidjabat's research interest is in molecular epidemiology of plasmids carrying antibiotic resistance genes and genetic context of antibiotic resistance genes.

Witchuda Kamolvit MD is a PhD student studying the genome of OXA-23 producing *A. baumannii* from Australia and Asian countries.

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Prof David Paterson's research interest includes study of the molecular and clinical epidemiology of infections with antibiotic resistant organisms. The focus of this work is the translation of knowledge into optimal prevention and treatment of these infections.

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Veterinary mycology is often a neglected field in veterinary medicine with many veterinarians treating infections empirically, or failing to send samples to diagnostic laboratories for identification. Few researchers undertake projects in this field. However, between 1968 and 1992 there was a major veterinary mycology laboratory in Australia. This laboratory was established at the Animal Research Institute, Yeerongpilly by Michael Desmond Connole known to friends and colleagues as "Des". Des was actively engaged in research, training, diagnosis and control of veterinary mycoses in Australia for nearly 50 years. He is an international figure in the field of mycoses and has over 45 publications including: "A review of animal mycosis in Australia", which was published in *Mycopathologia* in 1990. Des specialised in the isolation of fungi including dermatophytes, zygomycetes, pathogenic hyphomycetes and mycotoxin producers. This article honours Des's contribution to veterinary mycology in Australia and internationally.

Des had humble beginnings growing up in Camp Hill in Brisbane. He completed his junior pass at St Laurence's in South Brisbane and then entered the public service as a clerk in 1943. He completed his senior pass by studying nights and Saturday mornings at the Queensland Teacher's Training College in Edward Street, Brisbane.

In 1947, Des started working as a cadet science graduate (bacteriologist) at the Animal Research Institute (ARI) at Yeerongpilly (Figure 1) where he was supervised by Geoffrey Clive Simmons who was the chief bacteriologist and first graduate bacteriologist to be employed at ARI. In 1957, Des graduated from The University of Queensland with a Bachelor of Science, majoring in Bacteriology (Figure 2). Des worked as a diagnostic bacteriologist, from 1957–1963, identifying the causal agents (bacteria, viruses, protozoa and fungi) of animal diseases. During his spare time he was told to "work on mycology as there has been no work done on veterinary mycology". Thus, Des's great love of veterinary mycology began.

Des's first solo publication detailed a natural ringworm infection in the guinea pig colony at the ARI due to *Trichophyton mentagrophytes*. The paper describes the progression of lesions, and the diagnosis of the dermatophytes via microscopic examination and culture of skin scrapings and hair[1]. Much of Des's work involved the study of dermatophytes. In 1959, he identified *Trichophyton verrucosum* from a bovine skin scraping. This was the first time it had been isolated from cattle in Australia and the isolate was sent it to the London School of Hygiene and Tropical Medicine, to confirm his identification. Previously, *T. verrucosum* had only been isolated from human cases in Victoria, Australia. Humans with this dermatophyte infection worked on farms and with animals. Hence, the

Figure 1. Animal Research Institute (ARI) at Yeerongpilly, Queensland, Australia.

Figure 2. 1957, Des graduating from The University of Queensland with a Bachelor of Science, majoring in Bacteriology.

isolation of the agent from cattle was a major breakthrough and identified a zoonotic link. This led to a survey of bovine ringworm (1960–1962) where all field staff submitted suspected ringworm cases from bovines. Des was able to identify 32 strains of *T. verrucosum* from field specimens and 14 strains from stock at the institute. He identified that *T. verrucosum* was the usual agent of cattle ringworm and occurred in all parts of Queensland, in both dairy and beef cattle and while it could occur in all age groups, young cattle were most at risk[2].

In 1963, Des went on study leave to the University of Glasgow where he worked under Professor J. C. Gentles. Here he developed his skills in diagnostic mycology, teaching and research (Figure 3). During this period he studied dermatophytes and keratinophilic fungi of dogs and cats. This work led to two publications, the first describing the "hairbrush sampling" technique which he performed on 154 animals to detect the presence of keratinophilic fungi[3] and the second was published with Christine Dawson who continued his work, with some variations in culture methods and examined another series of animals at approximately the same time the following year[4]. The "hairbrush sampling" technique was identified as adequate if the fungus is present in quantity, as typically occurs in dermatophyte infections. On his way back to Australia from Glasgow, Des spent seven weeks at the Central Veterinary Laboratory, Weybridge, England, and visited other laboratories, universities and industries in the United Kingdom, visited the Pasteur institute, Paris and laboratories in the United States of America including the Centre for Disease Control, Atlanta, Georgia.

Figure 3. 1963, Des on study leave at the University of Glasgow.

On returning to Australia, Des published data from a survey of dermatophytes from dogs and cats. He was able to identify *Microsporum canis* and *M. gypseum.* This was the first record of *M. gypseum* infection in dogs in Australia[2]. In 1968, Des published the first record of a *T. mentagrophytes* infection in a dog in Australia[5].

In 1964, Des moved to Townsville where he worked as the diagnostic bacteriologist and mycologist at the Oonoonba Animal Health Station, until 1968. Here he developed expertise in identifying potentially toxic fungi (esp. *Aspergillus*) presented in animal feedstuffs[6].

In 1967, via Invitation of the Commonwealth Bureau of Animal Health, Des was asked to perform the first Australian review of animal mycoses[7]. At this time, mycotic diseases of animals were of economic importance and some as zoonoses were of public health interest. There was no satisfactory treatment for many mycotic infections and the epidemiology of mycoses was virtually unknown. Des with co-author L.A.Y. Johnston reviewed veterinary superficial and systemic mycoses, and mycotoxicoses from Australia[7].

In 1968, Des returned to ARI, as the Senior Bacteriologist (Mycology). In the mycology unit at ARI he diagnosed dermatophytes, hyphomycetes, zygomycetes, and examined feedstuffs for toxigenic fungi. He was sent fungal cultures from intrastate, interstate and overseas.

Throughout his career Des studied dermatophytosis in horses. Ringworm in horses is undesirable as horses with detectable infections are not allowed to race and infections are potentially transmissible to humans. Des was involved in the first isolation of *T. equinum* the usual ringworm agent affecting horses in Queensland in 1960–1963[2], but it was not until 1984 after an outbreak of severe ringworm in horses in the Oakey area, that this was recognised as a *Trichophyton equinum var. equinum* infection[8,9]. Des was also the first to isolate *Microsporum gypseum* from a skin scraping from a four year old pony from Central Queensland in 1966[10]. In 1974, during an outbreak of *M. gypseum* infections, Des with equine veterinarian Dr Reginald "Reg" Pascoe described a number of natural and experimental infections in horses[11]. This body of work described gross lesions, diagnosis, microscopic and cultural characteristics of dermatophytes, and the epidemiology of

infection including risk factors such as biting insects, and moist atmospheric conditions. They were able to implicate fomites like girth straps and saddle cloths in the transmission of infections[11].

Des also isolated *M. nanum* from pigs in Queensland and investigated experimental transmission of the dermatophyte[12]. Des performed a number of studies examining fungal metabolites to use as insecticides against the cattle tick (*Boophilus microplus*)[13] and the sheep blowfly (*Lucilia cuprina*)[14,15]. He also continued to investigate mycotoxins, and reported on a number of cases involving aflatoxicosis and isolation of mycotoxins in animal feeds[16–19].

Des also identified a number of rare opportunistic mycoses such as a *Drechslera rostrata* infection in a cow, which was first infection of this type recorded in Australia[20], and reported on mycotic nasal granumalomas in cattle from 1966–1975[21]. He identified *Conidiobolus incrongruus* as the cause of rhinocerbral and nasal granulomas in sheep[22]. He reported on equine phycomycosis[23] and identified *Moniliella suaveolens* as the cause of opportunistic granulomas in cats[24], which had not been previously identified from animals or man. He even helped identify green algae from green lymph nodes from cattle with lymphadenitis[25].

Des was the supervising bacteriologist at the ARI during 1974–1982 and continued his diagnostic work and also presented courses to medical mycology students, postgraduate students and trained veterinary mycologists from Australia and overseas. In 1982, on behalf of the Australian Development Assistance Bureau, Canberra, Des carried out a short-term consultancy in the Mycology Department of the Animal Disease Institute, Bogor, Indonesia. He spent time training technicians in mycology skills, in what was quite a challenging tropical climate, with many contamination problems, including fungi growing on microscope lenses.

In 1990, he was asked to perform a second review on animal mycoses in Australia. This review[26] covered literature on mycoses in animals in Australia since the last review published in 1967.

In addition to his outstanding work as a diagnostic bacteriologist, mycologist and researcher, Des played an important role in the Australian Society of Microbiology. He was a founding member and first Treasurer of the Queensland branch. In 1978, he became a foundation member of the Australasian Federation of Medical and Veterinary Mycology a special interest group of the ASM. He served on various committees and contributed to many conferences. In 1982, he received the first Churchill fellowship awarded in Australia in the field of medical and veterinary mycology and travelled to New Zealand to study developments in animal and human mycology. Des has also played an active role with the International Society for Human and Animal Mycology (ISHAM). He has attended 13 ISHAM congresses since 1964, including this year's meeting in Berlin. Des retired in 1992, after nearly 50 years of public service. He continued to work for a number of years delivering lectures, consulting and refereeing journal articles. Now he enjoys his retirement with his wife, Kathy, though he still regularly attends mycology conferences.

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References

1. Connole, M.D. (1963) Ringworm in guinea pigs due to *Trichophyton mentagrophytes. Qld J. Argic. Sci.* **20**, 293–297.
2. Connole, M.D. (1963) A review of dermatomycoses of animals in Australia. *Aust. Vet. J.* **39**, 130–134. doi:10.1111/j.1751-0813.1963.tb04252.x
3. Connole, M.D. (1965) Keratinophilic fungi on cats and dogs. *Sabouraudia* **4**, 45–48. doi:10.1080/00362176685190121
4. Gentles, J.C., *et al*. (1966) Keratinophilic fungi on cats and dogs. II. *Sabouraudia* **4**, 171–175. doi:10.1080/00362176685190391
5. Connole, M.D. (1968) Ringworm due to *Trichophyton mentagrophytes* in a dog. *Aust. Vet. J.* **44**, 528. doi:10.1111/j.1751-0813.1968.tb09019.x
6. Connole, M.D. and Hill, M.W. (1970) *Aspergillus flavus* contaminated sorghum grain as a possible cause of aflatoxicosis in pigs. *Aust. Vet. J.* **46**, 503–505. doi:10.1111/j.1751-0813.1970.tb09175.x
7. Connole, M.D. and Johnston, L.A.Y. (1967) A review of animal mycoses. *Vet. Bull.* **37**, 145–153.
8. Smith, J.M., *et al*. (1968) *Trichophyton equinum* var. *autotrophicum*; its characteristics and geographical distribution. *Sabouraudia* **6**, 296–304. doi:10.1080/00362176885190581
9. Connole, M.D. and Pascoe, R.R. (1984) Recognition of *Trichophyton equinum* var. *equinum* infection of horses. *Aust. Vet. J.* **61**, 94. doi:10.1111/j.1751-0813.1984.tb15527.x
10. Connole, M.D. (1967) *Microsproum gypseum* ringworm in a horse. *Aust. Vet. J.* **43**, 118. doi:10.1111/j.1751-0813.1967.tb08909.x
11. Pascoe, R.R. and Connole, M.D. (1974) Dermatomycosis due to *Microsporum gypseum* in horses. *Aust. Vet. J.* **50**, 380–383. doi:10.1111/j.1751-0813.1974.tb05342.x
12. Connole, M.D. and Baynes, I.D. (1966) Ringworm caused by *Microsporum nanum* in pigs in Queensland. *Aust. Vet. J.* **42**, 19–24. doi:10.1111/j.1751-0813.1966.tb04606.x
13. Connole, M.D. (1969) Effect of fungal extracts on the cattle tick, *Boophilus microplus. Aust. Vet. J.* **45**, 207. doi:10.1111/j.1751-0813.1969.tb01939.x
14. Blaney, B.J., *et al*. (1985) Fungal metabolites with insecticidal properties I. Relative toxicity of extracts of fungal cultures to sheep blowfly *Lucilia cuprina IWeid. Gen. Appl. Entom.* **17**, 42–46.
15. Green, P.E., *et al*. (1989) Identification and preliminary evaluation of viriditoxin, a metabolite of *Paecilomyces varioti* as an insecticide for sheep blowfly, *Lucilia cuprina* (Wied). *Gen. Appl. Entom* **21**, 33–37.
16. Connole, M.D., *et al*. (1981) Mycotoxins and mycotoxic fungi in Queensland stockfeeds 1971-80. *Aust. Vet. J.* **57**, 314–318. doi:10.1111/j.1751-0813.1981.tb05835.x
17. McKenzie, R.A., *et al*. (1981) Acute aflatoxicosis in calves fed peanut hay. *Aust. Vet. J.* **57**, 284–286. doi:10.1111/j.1751-0813.1981.tb05816.x
18. Blaney, B.J., *et al*. (1989) Aflatoxin and cyclopiazonic acid production by Queensland isolates of *Aspergillus flavus* and *Aspergillus parasiticus. Aust. J. Agric. Res.* **40**, 395–400. doi:10.1071/AR9890395

19. Ketterer, P.J., *et al*. (1975) Canine aflatoxicosis. *Aust. Vet. J.* **51**, 355–357. doi:10.1111/j.1751-0813.1975.tb15946.x
20. Pritchard, D., *et al*. (1977) Eumycotic mycetoma due to *Drechslera rostrata* infection in a cow. *Aust. Vet. J.* **53**, 241–244. doi:10.1111/j.1751-0813.1977.tb00197.x
21. McKenzie, R.A. and Connole, M.D. (1977) Mycotic nasal granuloma in cattle. *Aust. Vet. J.* **53**, 268–270. doi:10.1111/j.1751-0813.1977.tb00212.x
22. Ketterer, P.J., *et al*. (1992) Rhinocerebral and nasal zygomycosis in sheep caused by *Conidiobolus incongruus. Aust. Vet. J.* **69**, 85–87. doi:10.1111/j.1751-0813.1992.tb15556.x
23. Connole, M.D. (1973) Equine phycomycosis. *Aust. Vet. J.* **49**, 214–215. doi:10.1111/j.1751-0813.1973.tb06796.x
24. McKenzie, R.A., *et al*. (1984) Subcutaneous phaeohyphomycosis caused by *Moniliella suaveolens* in two cats. *Vet. Pathol.* **21**, 582–586.
25. Rogers, R.J., *et al*. (1980) Lymphadenitis of cattle due to infection with green algae. *J. Comp. Pathol.* **90**, 1–9. doi:10.1016/0021-9975(80)90023-7
26. Connole, M.D. (1990) Review of animal mycoses in Australia. *Mycopathologia* **111**, 133–164. doi:10.1007/BF02282798

Biography

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The Yersinia story: A proof of the Laurentia and Australia continents link

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Predominant virulent European *Yersinia enterocolitica* 4/O:3 produce two types of β-lactamases, enzyme A and enzyme B and belong to phage type VIII (4/O:3/VIII). *Y. enterocolitica* 4/O:3 isolated in Canada and Australia are identical producing only enzyme A and belonging to the same phage type IXb (4/O:3/IXb/A). Their failure to express enzyme B is due to same defect in *ampC* gene. Rare European *Y. enterocolitica* 4/O:3 strains lacking enzyme B all have the same defect in *ampC* gene which is different from that observed in Canadian and Australian strains. The difference in defective *ampC* gene between European and Canadian/Australian 4/O:3 shows that Canadian/Australian 4/O:3 are not European 4/O:3 that are defective in the expression of enzyme B. The presence of the predominant and unique *Y. enterocolitica* subtype 4/O:3 at two areas far apart on the earth can only be explained by a common geographical origin. This provides a microbiological proof of linkages between Paleoproterozoic and Mesoproterozoic geological features in north western Canada and Australia presenting a new insight and perspective to both Geology and Microbiology.

The bug

Yersinia pestis, Yersinia pseudotuberculosis and Yersinia enterocolitica are the three most important species bacteria of the genus *Yersinia* named after the Swiss-born French physician and bacteriologist Alexander Yersin. Dr Alexander Yersin was sent to Hong Kong by Institut Pasteur, Paris and French government to investigate the bubonic plague that was sweeping through China in 1894. The bacteriologist isolated the bacillus, *Y. pestis*, from both rodents and affected patients linking the illness to the intermediate host. *Y. pseudotuberculosis* causes serious illness in young cattle while *Y. enterocolitica* mainly causes gastro-enteritis in human and more serious infections in susceptible patients. *Y. enterocolitica* is heterogeneous and of the six biotypes 1A, 1B, 2, 3, 4 and 5, biotype 1A is non-pathogenic. Among pathogenic *Y. enterocolitica*, biotype 4 serotype O:3 is the predominant virulent bio-serotype worldwide.

Unlike *Y. pestis* and *Y. pseudotuberculosis*, *Y. enterocolitica* is known to produce two chromosomal or inherent β-lactamases, the enzymes that hydrolyse β-lactam antibiotics such as penicillin and more active agents of that group. The two β-lactamases are the broad spectrum enzyme A belonging to molecular class A and the enzyme B, an inducible cephalosporinase of class C[1–4]. The degree of enhancement of enzyme B by an inducer varies substantially with each biotype of *Y. enterocolitica* while it is consistent within each of the sub-types[4–7]. The induction of enzyme B in European *Y. enterocolitica* 4/O:3, all belonging to phage type VIII (4/O:3/VIII), the predominant virulent bio-serotype-phage type isolated in Europe and many parts of the world is low and negative in disc diffusion induction[6,7]. Canadian *Y. enterocolitica* 4/O:3/IXb strains of sub-type A, fail to show induction of β-lactamase. Their lack of induction is due to the absence of the inducible enzyme B[6,7]. Coincidentally, all *Y. enterocolitica* 4/O:3 isolated in Australia also fail to express enzyme B[3–7].

The investigation

Over 200 *Y. enterocolitica* 4/O:3 isolated in Australia and Canada were checked for biotype, serotype and phage type. They were also examined for the β-lactamase expression by disc diffusion method using amoxicillin-clavulanate 3 µg (AMC 3) and ampicillin 25 µg (AMP 25) discs and induction of enzyme B by disc diffusion[7,8].

All but one strain of *Y. enterocolitica* 4/O:3 isolated in Australia were found to show synergy between AMC 3 and AMP 25 discs revealing the presence of enzyme A and an inhibitory zone around AMC 3 with a diameter of 16 mm ± 2 mm, consistent with the absence of enzyme B (Fig. 1A)[6–8]. The remaining *Y. enterocolitica* 4/O:3 showed the presence of both enzyme A and enzyme B (Fig. 1B). This organism was isolated from the blood culture of a thalassemic patient returning to Australia after visiting relatives in Greece for three months. The isolate was sent to WHO collaborating centre

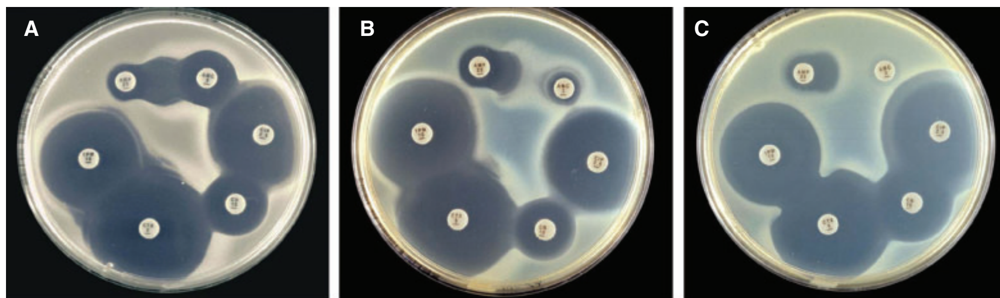

Figure 1. Routine CDS method of Antibiotic Susceptibility Testing (AST)[9].
(a) Australian *Y. enterocolitica* 4/O:3/IXb/A showing a small zone around ampicillin disc (AMP 25) and a larger zone up to 18 mm in diameter around Augmentin disc (AMC 3), characteristics of this subgroup of *Y. enterocolitica.* The dark area between AMP 25 and AMC 3 discs illustrates the marked synergy between the two antibiotics reflecting the exquisite susceptibility of the organism to the combination amoxi-clavulanate i.e. Augmentin.
(b) European *Y. enterocolitica* 4/O:3/VIII showing a narrow zone around Augmentin disc (AMC 3) illustrates the presence of enzyme B.
(c) Canadian *Y. enterocolitica* 4/O:/IXb/AB. Strains of this sub-group 4/O:/IXb/AB produce both enzyme A and a highly inducible enzyme B showing a flattened CTX 5 zone near IPM 10 disc.

for Yersinia in Paris for phage typing (IP 24215), and was found to belong to phage type VIII, the European phage type known to produce both enzymes A and B[6]. The European sub-type 4/O:3/VIII of the isolate was consistent with the patient's history of travelling to Europe and explained the presence of an European sub-type among the 87 strains of *Y. enterocolitica* 4/O:3 isolated in Australia. Australian *Y. enterocolitica* 4/O:3 had been previously phage typed, courtesy of WHO collaborating centre for Yersinia, Paris. They were found to belong to phage type IXb, the so-called Canadian phage type. Thus, without exception, all local Australian *Y. enterocolitica* 4/O:3 isolates examined were identical to Canadian *Y. enterocolitica* 4/O:3 phage type IXb that produced only enzyme A i.e. they were all of sub-type 4/O:3/IXb/A[6].

Initial examination of 123 strains of *Y. enterocolitica* 4/O:3 isolated in Canada over a period of six years showed that all strains isolated in 2002 and 2003 and the majority of strains isolated in 2004–2007 belonged to phage type IXb, confirming that phage type IXb was typical of local Canadian *Y. enterocolitica* 4/O:3 (Table 1). The results of enzyme A and enzyme B detection showed that the majority of isolates failed to express enzyme B (Fig. 1A), thus belonged to subtype 4/O:3/IXb/A[6]. They were therefore identical to local Australian 4/O:3/IXb/A. There was also a small number of phage type IXb isolates that produced both enzyme A and enzyme B i.e. belonging to sub-type 4/O:3/IXb/AB (Table 1). These isolates had no zone around AMC 3 disc, therefore consistent with a high amoxi-clav MIC of 32 mg/L[6]. Canadian 4/O:3/IXb/AB isolates in this series were also positive in induction by disc diffusion test with the typical flattened zone of cetotaxime 5 µg (CTX 5) near imipenem 10 µg (IMP 10) disc, illustrating the presence of a highly inducible enzyme B (Fig. 1C)[6,7]. From 2004 to 2007, for each year, two to three isolates were of phage type VIII (Table 1). They produced both enzymes A and B and showed no flattened CTX 5 zone near IPM 10 disc i.e. negative in induction by disc diffusion test. The lack of the flattened CTX 5 zone near IPM 10 disc reflected the low induction of enzyme B, identical to European 4/O:3/VIII illustrated (Fig. 1B)[6]. These strains were therefore likely to have been brought to Canada

Table 1. *Y. enterocolitica* 4/O:3 isolated in Canada from 2002 to 2007.

Year (number of strains)	Sub-type 4/O:3/IXb/A Enzyme A alone	Sub-type 4/O:3/IXb/AB Enzyme B present Disc induction positive	Sub-type 4/O:3/VIII Enzyme B present Disc induction negative
2002 (21)	20	1	0
2003 (19)	18	1	0
2004 (17)	15	0	2
2005 (18)	13	2	3
2006 (24)	20	2	2
2007 (24)	19	2	3

by travellers from Europe, similar to the case of strain IP 24215 isolated in Australia.

The similarity between the predominant *Y. enterocolitica* 4/O:3 IXb/A isolated in Australia and Canada, currently two geographically distant parts of the earth is supported by other evidence. Not only do they belonged to the same phage type IXb and failed to express enzyme B due to an inactive *ampC* gene[10], their behaviour at molecular level was also identical and in the same token, differed from that of European *Y. enterocolitica* 4/O:3/VIII. When de la Prieta and colleagues investigated the defective *ampC* gene in *Y. enterocolitica*, they examined four unusual European *Y. enterocolitica* 4/O:3/VIII strains that failed to express the enzyme B along with two Australian 4/O:3/XIb/A and two Canadian 4/O:3/XIb/A. In all four unusual European 4/O:3/VIII strains examined, they found a mutation at position 2298, which introduced an A to G change at that position, leading to the substitution of a threonine with an alanine at position 252, causing a misreading of the protein, thus the failure to produce enzyme B[11]. On the other hand, with the two Canadian and two Australian strains of *Y. enterocolitica* 4/O:3/IXb/A, they found that the lack of expression of enzyme B was due to the same 2 base pair (bp) deletion, CG at position 755-756 of *ampC* gene. The deletion caused a change in the reading frame resulting in the misreading of the protein after amino-acid 252 and a premature stop thus the failure to express enzyme B.

The insight

When an unusual European 4/O:3/VIII strains fails to express enzyme B, it is invariably due a mutation at position 2298 on *ampC* gene. By contrast, all four Australian and Canadian 4/O:3/IXb/A examined have a 2 base pair deletion, CG at position 755-756 causing the misreading of the protein after amino-acid 252 and the failure of enzyme B expression. These findings prove that Australian and Canadian *Y. enterocolitica* 4/O:3/XIb/A are not *Y. enterocolitica* 4/O:3/VIII that had undergone a mutation leading to the lack of expression of enzyme B and at the same time had become susceptible to bacterio-phage IXb instead of VIII. Such distinctive difference at molecular level for the failure to express enzyme B between European 4/O:3/VIII and Canadian and Australian 4/O:3/IXb/A demonstrates that *Y. enterocolitica* 4/O:3/IXb/A are not of European origin[12].

That insight raises the interesting question concerning the origin of the unique and predominant *Y. enterocolitica* of subtype *Y. enterocolitica* 4/O:3/IXb/A isolated in Canada and Australia, two areas far apart on the earth. The likely and perhaps only possible explanation is that they share the same geographic origin. That origin would date at a period before the drifting of the continents[13]. The presence of a unique and identical micro-organism found only in Canada and Australia supports the hypothesis that the Pacific Margin of Laurentia and East Antartica-Australia is a conjugate rift pair[13].

The presence of a low number of *Y. enterocolitica* 4/O:3 phage type XIb producing an enzyme A and a highly inducible enzyme B .i.e. *Y. enterocolitica* 4/O:3/XIb/AB amongst the 123 Canadian 4/O:3/XIb strains adds another layer to the origin and the evolution of Canadian strains of *Y. enterocolitica* 4/O:3/IXb. These highly inducible Canadian 4/O:3/XIb/AB (Fig. 1C) have never been reported elsewhere except in Canada. The presence of a low number of *Y. enterocolitica* sub-type 4/O:3/IXb/AB with a highly inducible enzyme B, isolated only in Canada and nowhere else in the world, suggests that in fact, they are most likely descendants of an enzyme B producer mutant with no defect in *ampC* gene. The mutant must have arisen from the original and predominant *Y. enterocolitica* sub-type 4/O:3/XIb/A after the drifting of the continents. These observations present another argument against the presumptive European origin for Canadian and Australian *Y. enterocolitica* 4/O:3/IXb[12].

The observations compiled in this investigation provide a new insight to both Microbiology and Geology along with a new perspective to the age and origin of micro-organisms which inhabited the earth long before the drifting of the continents[13–15].

Author’s note

This article is an extract from the book of the same title, *The Yersinia story: A proof of the Laurentia and Australia continents link* (Lambert Academic Publishing) available at Amazon.com.

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References

1. Cornelis, G. (1975) Distribution of β-lactamase A and B in some groups of *Yersinia enterocolitica* and their role in resistance. *J. Gen. Microbiol.* **91**, 391–402. doi:10.1099/00221287-91-2-391
2. Pham, J.N., *et al*. (1991) Biotype and antibiotic sensitivity of 100 clinical isolates of *Yersinia enterocolitica*. *J. Antimicrob. Chemother.* **28**, 13–18. doi:10.1093/jac/28.1.13
3. Pham, J.N., *et al*. (1991) A study of the β-lactamases of 100 clinical isolates of *Yersinia enterocolitica*. *J. Antimicrob. Chemother.* **28**, 19–24. doi:10.1093/jac/28.1.19
4. Pham, J.N., *et al*. (1992) β-lactamase induction by imipenem in *Yersinia enterocolitica*. *Path.* **24**, 201–204. doi:10.3109/00313029209063174

5. Pham, J.N., *et al*. (2000) The β-lactamases and β-lactam antibiotic susceptibility of *Yersinia enterocolitica*. *J. Antimicrob. Chemother.* **46**, 951–957. doi:10.1093/jac/46.6.951

6. Pham, J.N., *et al*. (1995) Susceptibility to β-lactam agents of *Yersinia enterocolitica* biotype 4, serotype O:3 isolated in various parts of the world. *J. Med. Microbiol.* **43**, 9–13. doi:10.1099/00222615-43-1-9

7. Pham, J.N., *et al*. (1993) The detection by a disc diffusion technique of inducible β-lactamase in *Yersinia enterocolitica*. *J. Antimicrob. Chemother.* **31**, 1004–1005. doi:10.1093/jac/31.6.1004

8. Pham, J.N., *et al*. (1999) The detection of enzyme A of *Yersinia enterocolitica* by a disc diffusion method. *Path.* **31**, 268–270. doi:10.1080/003130299105115

9. Bell, S.M. *et al*. (2012) Antibiotic susceptibility testing by the CDS method. A Manual for Medical and Veterinary laboratories, Modified Sixth Edition, Sept 2012. Link for web site: http://web.med.unsw.edu.au/cdstest.

10. de la Prieta, M.C., *et al*. (1995) β-lactamase genes and β-lactam antibiotic susceptibility in *Yersinia enterocolitica*. *Contrib. Microbiol. Immunol.* **13**, 184–187.

11. de la Prieta, M.C., *et al*. (2006) Characterisation of defective β-lactamase genes in *Yersinia enterocolitica*. *J. Antimicrob. Chemother.* **58**, 661–664. doi:10.1093/jac/dkl267

12. Neubauer, H., *et al*. (2000) *Yersinia enterocolitica* 16S rRNA gene types belong to the same genospecies but form three homology groups. *Int. J. Med. Microbiol.* **290**, 61–64. doi:10.1016/S1438-4221(00)80107-1

13. Burrett, C., *et al*. (2000) Proterozoic Australia-Western United States (AUSWUS) fit between Laurentia and Australia. *Geol.* **28**, 103–106. doi:10.1130/0091-7613(2000)28<103:PAUSAF>2.0.CO;2

14. Thornkelson, D.J. *et al.*, (2001) Early Proterozoic magmatism in Yukon, Canada: constraints on the evolution of northwestern Laurentia. *Can J Earth Sci/Rev Can Sci Terre.* **38**, 1479–1494.

15. Betts, P.J., *et al*. (2008) Comparing 1800–1600 Ma accretionary and basin processes in Australia and Laurentia: possible geographic connections in Columbia. *Precambrian Res.* **166**, 81–92. doi:10.1016/j.precamres.2007.03.007

Biography

Jeanette Pham is the Senior Scientist in charge of the CDS method of Antibiotic Susceptibility Testing Reference Laboratory, Sydney, Australia. Her research interests are mechanisms of resistance to β-lactam antibiotics and the β-lactamases of Gram-negative bacteria especially those of *Y. enterocolitica*.

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Tribute to Nancy Millis

Jim Pittard

Professor Emeritus Nancy Millis AC.MBE.FAA.FTSE, died on the 29th of September at the age of 90. Nancy's long life had been filled with exemplary service over a broad range of activities affecting her discipline Microbiology, Higher Education and the wider community.

She graduated Bachelor of Agricultural Science from Melbourne University in 1945. Because of her father's illness she had been obliged to leave school early and had completed Matriculation at night school over a period of two years. In those days the Faculty of Science would not accept applicants who had taken more than one year to complete their Matriculation, hence Nancy's enrolment in Agricultural Science. As it turned out this extraordinarily rigid attitude of the Faculty turned out to be a godsend for generations of Agricultural Science students who were taught by Nancy in subsequent years. Nancy thoroughly enjoyed her Agricultural Science course including her time at Dookie. She once said that she always felt at home in her Gum Boots. After her undergraduate course she enrolled for a Degree of Master of Ag. Science with Vic Skerman studying a strain of Pseudomonas able to reduce nitrate. This was the beginning of extensive research carried out by Nancy on the microorganisms involved in the nitrogen cycle. After completing her Master's she accepted a position with the Department of Foreign Affairs to work in New Guinea studying the Agricultural practices of the local women. Unfortunately for Nancy she had not been there very long before she succumbed to a massive intestinal infection, which almost killed her. After some emergency surgery and three months in Port Morseby she was transported via Brisbane to Melbourne where over many months with careful nursing and antibiotics she slowly regained her health. Not wishing to return to the tropics and coming upon an advertisement in the paper for a PhD scholarship offered by Boots for study at Bristol University in the UK, Nancy sent off an application and with a small dowry that she had received from her aunt she took herself off in the hope of a successful outcome. She was granted the scholarship and a PhD position and completed a PhD on the micro-organisms causing spoilage of Cider Fermentations in three years. After returning to Melbourne, she tried unsuccessfully for jobs at Carlton and United Breweries and at Kraft but she was probably not male enough for one and considered over qualified for the other. Fortunately, Syd Rubbo who was Head of the then Bacteriology Department at the University of Melbourne was quick to appreciate her qualities and her skills. He appointed her as Senior Demonstrator in 1952, she was promoted to Lecturer one year later, to a Reader in 1968 and to a Personal Chair in 1982. In 1954 Syd organised an early sabbatical, which enabled her, supported by a Fulbright fellowship and a scholarship offered by the American Society of University Women in Madison Wisconsin to join the laboratory of Marvin J Johnson where she studied the latest developments in the fermentation involved in the production of Penicillin. Back in Melbourne after her study leave she started her own research using strains of *Aspergillus niger* to produce Citric Acid. At the same time she was involved in lecturing to both the science students and the Ag. Science students.

Her next Sabbatical was taken in 1963 when she attended C.B. Van Niel's famous course in General Microbiology given at Hopkins Marine Station followed by a nine month period at The Institute of Applied Microbiology at Tokyo University working with Professor Suichi Aiba on methods of continuous culture of micro-organisms.

During this visit, Nancy, Professor Aiba and a visiting scientist Professor Arthur Humphrey together delivered a course in what was termed Biochemical Engineering. As Nancy later remarked this was the first integrated course in Biotechnology to be given in Japan. On her return to Melbourne Nancy collated these lectures into a textbook "Biochemical Engineering". This was one of the first textbooks in the brave new world of Biotechnology and is still being recommended in some courses in Chemical Engineering today some fifty years after its first publication.

One of the most extraordinary aspects of Nancy's academic career was her breadth of knowledge about all aspects of Microbiology and its application. Her research interests ranged from bacteriophage and bacteriocins of rumen bacteria, to micro-organisms involved in the nitrogen cycle in marine sludges, to bacteria able to break down phenols and various hydrocarbons. She also investigated the possibility of using hydrocarbons as a food for growing yeasts and she was constantly being asked to solve problems caused by the growth of micro-organisms in unexpected places and on unexpected substrates. One of these involved deterioration of a major Highway between Melbourne and Sydney and another the blockage of drainage pipes in the new Art Centre in Melbourne. This breadth of understanding coupled with a healthy scepticism for any

unsubstantiated claims made her a very popular and very successful teacher. She was able to engage with students whether in field work, in the lab or in the lecture theatre. She is fondly remembered by generations of Ag. Science and other students at Melbourne University. In addition to the Ags, Nancy introduced and taught one of the first courses to be offered in Australia on Industrial Microbiology. She was also involved for many years giving lectures on this topic to the Chemical Engineering students.

Her deep understanding of Industrial and Agricultural Microbiology combined with a no nonsense approach to solving important problems meant that she was ideally placed to help steer the new developments in molecular genetics into a safe and acceptable framework for application in both Industry and Agriculture. In 1978 Nancy was a member of the Fenner committee reviewing Recombinant DNA in Australia for the Academy of Science. As a result of that report the government set up a new committee The Recombinant DNA Monitoring Committee (RDMC) with Nancy as the Chair. During its eight year tenure this committee, under Nancy's guidance produced and oversaw the implementation of important guidelines for work in Laboratories, in Industry and for the Planned Release of Genetically Modified Organisms. Due to an Acronym change this committee became the Genetic Manipulation Advisory Committee (GMAC) and Nancy continued as Chair until, this committee was replaced by the Office of the Gene Technology Regulator (OGTR). The relatively untroubled and careful introduction of this Technology in Australia owes much to the dedication and skills of Nancy Millis in her interactions with government, scientists and the general public.

Nancy also had an abiding interest in water quality and water management. She was Chair of the Board for the CRC for Water Quality and Treatment, a member of the Board of the CRC for Fresh Water Ecology, Chair of the Research Advisory Committee Murray –Darling Freshwater Research Centre and member of the Board of MMBW. At the same time her own research interests extended to the microbial ecology of wetlands and estuaries and involved pollution studies in Western Port and Port Phillip Bays.

From its very foundation Nancy was an enthusiastic and prominent supporter of the Australian Society for Microbiology. She was National Secretary from 1964–67, President 1978–80, Rubbo Orator 1982 and made an Honorary life Member of the Society in 1987. She was very much involved in the Annual Scientific Meetings where her wit and wisdom were greatly appreciated.

Nancy's very effective contributions as a committee member meant that during her lifetime she served with distinction on many other committees too numerous to list here. She was generous with her time and always conscientious in her preparation. Her consistent contributions have been recognised with a number of accolades. In 1977 she was made a Member of the British Empire (MBE), and in 1990 was made a Companion of the Order of Australia (AC). She was elected to the Academy of Technological Sciences and Engineering (FTSE) in 1977 and to the Academy of Sciences (FAA) in 2004 by special election recognising her conspicuous service to the cause of science with her outstanding career in Microbiology. In 2002 she was one of five scientists immortalised on stamps by the Australia Post as living legends. In 1982 she was appointed to a Personal Chair at the University of Melbourne, being amongst the first women to receive this appointment. In 1987, after her retirement, she was appointed Professor Emeritus. She received an Honorary DSc and an Honorary LLD from the University of Melbourne and an Honorary DSc from La Trobe University. Between 1992 and 2006 she was Chancellor at La Trobe University. She has a number of lectures and scholarships named after her, the Nancy Millis lecture established by La Trobe University, the Millis Oration by AusBiotech, the Nancy Millis Agriculture student scholarship at the University of Melbourne, the Millis-Colwell award of the ASM. There is the Nancy Millis building, Murray Darling Freshwater Research Centre, Albury/Wodonga La Trobe University, the Nancy Millis laboratory University of Melbourne.

Above all, there are the collected memories of all those who had the pleasure and the privilege of working with Nancy over her lifetime and these will ensure that her life and her many, many contributions to the profession and to society will not be forgotten.

For more information about the life of Nancy Millis see S. Morrison. Interview with Professor Nancy Millis, Interview with Australian Scientists, Australian Academy of Science, 12 February 2001. http://w.w.w.science.org.au/scientists/interviews/m/nm.html

S. Morrison The Pick of the Crop. Proceedings of the Royal Society of Victoria 15.9.2010 Transactions I–XII.

S. Morrison, "Nancy Millis Microbiology Boots and All" in Farley Kelly (ed). On the Edge of Discovery; Australian Women in Science, Text Publishing Company, Melbourne, 1993, pp.155–177.

MICROBIOLOGY AUSTRALIA Guidelines for authors, 2013

***Microbiology Australia* has a very large and broad readership. It is released to science writers for further communication to public. Articles should be written in a style that is attractive to this general audience while keeping your peer group informed of the latest developments and their impact.**

The ideal article:

- **offers new information to scientists within and outside your own field of expertise:**
- **should be interesting and easy to read;**
- **discusses significance primarily but doesn't lack evidence;**
- **should be concise and contain good graphics.**

Articles

For all articles the opening paragraph should be an abstract in bold type and include the major points being made. State your conclusions up front, then discuss how they were arrived at. Concentrate almost entirely on the significance of the work being reported, rather than reporting detailed results.

'In Focus' articles

'In Focus' articles - major, review-type articles on a theme chosen by the Editorial Board and which should attract interest and understanding from those in all disciplines of microbiology - will be published in each issue of the journal, should be about **1500 words** in length and include 2-3 graphics or colour pictures.

You can discuss your own work in the context of other important work undertaken in the same field. It is important to acknowledge other work, since it helps paint a broader picture of your subject.

'Under the Microscope' articles and 'Laboratory reports'

These are short reports of between **500** and **1000 words** dealing with a current aspect of the topic. A graphic is welcomed.

Suggestions for cover photos are welcomed.

Preparation and format

All articles should be sent to the Editorial Team of *Microbiology Australia*, Ian, Jo and Hayley Macreadie as email attachments [editorasm@gmail.com].

Authors should follow the format outlined below when preparing articles.

- **Title page and author contact:** include your postal address, Tel, Fax and E-mail address. Add first and surnames of all authors. A headshot photo of authors is highly desirable.
- **Article heading:** this should be no longer than six words, and designed to catch the reader's eye.
- **Introductory paragraph:** this should be a *Nature*-style opening paragraph that doubles as an abstract. It should be in bold type and may be submitted for press releases (please advise if you do not wish your article to be available to the media).
- **References** should be numbered as a superscript as they appear in the text, while those appearing in tables or figure legends are to be numbered according to the position at which that table or figure is cited in the text. References are set out in the following style including title of the paper: up to two authors are listed; for more than two list the first followed by *et al*. Both first and last page numbers are given. [For authors using citation managing tools follow the style of "*Trends in Microbiology*".] e.g.

1 Fisher-Hoch, S.P. *et al*. (1985) Pathophysiology of shock in a fulminating viral infection. *J. Infect. Dis.* 152: 887–894.
2 Groseth, A. *et al*. (2005) Hemorrhagic fever viruses as biological weapons. In *Bioterrorism and Infectious Agents* (Fong, I. and Alibek, K., eds), pp. 169–187, Plenum Press.
3 Personal communications, unpublished observations and manuscripts in preparation should be round-bracketed and italicised in the text; for example: (*personal communication*).

- **Abbreviations and acronyms** should be used sparingly and must be clearly identified the first time they are used.
- **Figures** should be numbered as they appear in the text.
- **Acknowledgements** should be kept brief and are to appear immediately after the text.
- **Biographies** should contain a few lines on each author. e.g. Ian Macreadie is an Associate Professor of RMIT University. His research interests are in yeast molecular biology and its applications to address Alzheimer's disease. Bios will appear at the end of the article.

Graphics

Please try to keep within the word limit. If you have a lot of information to present consider the use of a summary table or graph. Also consider a hyperlink to a YouTube video or other on-line resources.
Please supply all pictures at **300dpi**, CMYK, actual size in .jpeg, .tiff or .eps format. Graphs and figures can be supplied in powerpoint.

Review process

Soon after submitting an article, you will receive notification of its receipt. Articles are reviewed by two reviewers: you are encouraged to suggest reviewers. Reviewers check the article's suitability for the journal and how the content has been communicated. If any part of the text seems problematic and needs rewriting, this will be discussed with the author. For the reviewing process we allow about 4 weeks.
Pdfs of the entire the issue will be sent to corresponding authors about 2 weeks before release of the issue. If you are planning to be away at this time, please include details of an alternate contact. Any corrections required should be promptly reported. A pdf with corrections will be supplied before publication.

Why timing is very important!

Thematic articles are coordinated to appear in a particular issue. Therefore please adhere to schedules to avoid delays in publication. Early on-line electronic issues appear in mid-February, mid-April, mid-August and mid-October with the print edition being mailed in the first week of the following month.

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