THE ends of the 19th and 20th centuries were golden periods of discovery and innovation in veterinary microbiology and were particularly significant periods in equine infectious disease research. Over its 125 year history, Veterinary Record has witnessed the birth of veterinary microbiology and the initial rapid progress made in the identification of the microbes responsible for infectious diseases in animals, especially in horses and farm animals. This first golden period was followed by great leaps forward in understanding bacterial and viral pathogenesis, followed by the discovery, use and then overuse of antimicrobials. Initial hopes of an end to bacterial disease in humans and animals were replaced over the four decades from the 1980s with the realisation that microbial disease is here to stay, and that 3.5 billion years of microbial evolution has equipped microbes with sophisticated survival mechanisms and a propensity for rapid genetic and phenotypic change that makes them easily capable of out-competing human interventions.

The new millennium heralded the second golden period in our knowledge of equine infectious diseases, with the genomic age transforming our understanding of bacteria and viruses to a level previously unimaginable and opening up new areas of discovery and innovation in pathogenesis, therapy, diagnostics and vaccine development.

Microbiology began as a ‘One Health’ discipline, although the term had not been coined in 1888, and, 125 years on, One Health has brought veterinary and equine infectious diseases back to their roots by recognising the interdependence of microbial disease and therapy in animals and people. Equine infectious disease is an exemplar of this story that began with glanders in 1888 and continues with Hendra virus in 2013. Throughout the history of veterinary infectious disease research, equine infectious diseases have been at the forefront of scientific discovery. This article will review some of the key events in equine infectious disease since 1888 and consider the challenges to come.

Microbiology at the close of the 19th century

In 1888, microbiology was just coming of age through the work of the founders of modern microbiology, the French chemist Louis Pasteur and the German physician Robert Koch (Mendelsohn 2002). Pasteur had recently developed his germ theory of disease and Pasteur and Koch’s independent work on anthrax in the 1870s led to the first coherent understanding of bacterial disease pathogenesis and bacteria as aetiological agents of disease. From his work on anthrax, Koch formulated a set of founding microbiological principles that persist to this day (Blevins and Bronze 2010), although recent developments in microbial genomics and ecology are challenging some aspects of these principles. Koch’s postulates, published in 1890, set out a logical framework for microbial disease pathogenesis studies by requiring that the microbe must be present in all diseased animals but not found in healthy individuals, that the microbe must be isolated in pure culture, and that, following inoculation of healthy animals, the disease must develop from the diseased animals and that, following inoculation of healthy animals, the disease must develop. The microbe must be isolated in pure culture, and that, following inoculation of healthy animals, the disease must develop from the diseased animals and that, following inoculation of healthy animals, the disease must develop from the diseased animals.

The pictures on the right compare the first issue of Veterinary Record, published on July 14, 1888, with how it looks today.
anthrax, typhoid, tuberculosis and cholera (Dunlop and Williams 1996).

**Glanders, strangles and African horse sickness**

The 1880s were a great era for equine infectious disease research, with several important equine infectious diseases (glanders, strangles and African horse sickness) taking centre stage alongside microbiologically important medical diseases. In an excellent example of One Health medicine, glanders and faccy, a zoonotic plague disease of horses that had been written about since Greco-Roman antiquity, was identified as having a bacterial cause when Loeffler and Schuetz identified the Gram positive rods of *Burkholderia mallei* (initially *Bacillus mallei* and later *Pseudomonas mallei*) in pus in 1882, and then showed it met Koch’s postulates. From Loeffler and Schuetz’s initial work, there was rapid progress, with development of a diagnostic test based on mallein (produced by Helman in St Petersburg in 1890 following the same methods Koch had used to produce tuberculin), which was first used as an intradermal skin test by Kalning in 1891 to identify infected horses. Glanders control measures were introduced across Europe between 1880 and 1893 with the mallein test greatly assisting disease control and eradication (Blancou 2000a).

The year 1888 was a particularly significant year for equine bacteriology. The genus *Streptococcus* had first been reported in the early 1880s by Rosenbach, and in 1888 *Streptococcus equi* was isolated as the causal agent of strangles by Schuetz (Blancou 2000b). *Streptococcus equi* has the scientific distinction of being not just one of the earliest identified animal bacterial pathogens, in the second golden period for microbiology at the end of the 20th century, it became the first purely veterinary pathogen to be genome sequenced (Holden and others 2009), triggering a new surge in strangles research and discovery (Harrington and others 2002, Waller and Jolley 2007).

This great wave of microbiological discovery was not confined to bacteria; the close of the 19th century also saw the birth of virology, with the identification of African horse sickness as one of the first animal diseases to be caused by a filterable agent smaller than bacteria. The breakthrough had come in 1892 when Ivanovskii showed that porcine bacteriologist filters were unable to prevent transmission of tobacco mosaic disease by filtered sap taken from infected plants.

In 1898 Loeffler and Frosch successfully transmitted foot-and-mouth disease using filtered vesicle fluid from infected cattle, providing the first evidence that this was an infectious disease caused by a filterable agent – a virus. John McFadyean, by now professor of pathology and Dean of the Royal Veterinary College, immediately picked up on the discovery of Loeffler and Frosch and, in 1900, using bacterial filters, showed that African horse sickness could be transmitted by bacteria-free, filtered blood and pericardial fluid from horses that had died of the disease (Cotchin 1990, Blancou 2000a).

**Globalisation and climate change**

From these beginnings 125 years ago, the equine infectious diseases story has been one of the spread of endemic and emerging diseases as the horse industry has globalised, superimposed with the effects of climate change on the distribution and ecology of vectorborne diseases. Recognition of the importance of equine infectious diseases to the global industry has seen the inclusion of African horse sickness in OIE List A (diseases with rapid spread and major economic importance) and several others in OIE List B (diseases with significant economic importance). In the UK a number of equine diseases are notifiable to Defra. Some of these have never occurred in the UK (for example, African horse sickness), others have not occurred for almost a century (for example, glanders), and others continue to occur periodically (for example, equine infectious anaemia, contagious equine metritis and equine viral arteritis).

**Timelines for key equine infectious diseases**

African horse sickness has remained endemic to sub-Saharan Africa and southern Africa with occasional incursions into the Middle East (1959-63), Spain (1966, 1987-90), Portugal (1899) and Yemen (1997) associated with the movement of infected equids, especially Old World equids such as zebra. Although African horse sickness has, thus far, remained geographically limited, the closely related *Ophiovirus*, bluetongue, spread progressively northwards through the Mediterranean basin in the 1970s and 1980s as its main *Culicoides* vector was able to extend its range through the effects of climate warming (Mellor and Hamblin 2004). The sudden appearance of bluetongue virus in Europe in 2006 was a wake-up call to the equine industry to be vigilant and prepared for incursions of exotic diseases. In the UK, a joint Defra/equine industry working party led by the Horse Trust started work on a control plan for the UK, which culminated in the adoption of the African Horse Sickness (England) Regulations by Parliament in November 2012.

Glanders has been eradicated from many regions since the mallein test was introduced but remains a threat to the global industry because it continues to be endemic in the Middle East, Asia, Africa and in some South American countries (Khan and others 2013).

Equine infectious anaemia has been recognised since the early 1900s and was another equine infectious disease shown to be caused by a filterable agent in those early days of discovery. The breakthrough in the control of equine infectious anaemia came in 1972 when Leroy Coggins published a method for serological diagnosis of infected horses by an immunodiffusion test, known to this day as the Coggins test (Coggins and others 1972). The disease continues to be endemic in many countries around the world, including the USA and some European countries. Awareness of the risks posed by the virus to the European horse industry was significantly raised following an outbreak in Ireland in 2006 (More and others 2006), the accession of Romania, an endemically infected country, to the EU and then the outbreaks of disease recorded in the UK and elsewhere in Europe since 2010. The disease has spread through the movement of infected horses and equine biological products, including plasma, and is a good example of how movement and trade spread equine infectious diseases.

Equine influenza virus was first isolated in Poland in 1956 and H7N7 virus circulated widely over the next two decades until its apparent disappearance in 1979. The first H3N8 equine influenza virus was isolated in North America in 1963 and, since then, H3N8 viruses have spread throughout the world, establishing endemic disease in all horse-keeping countries except Australia, New Zealand and Iceland.
an excellent example of virus evolution and genetic and antigenic change, the H3N8 viruses rapidly diverged into European and American lineages in the late 1980s, with both virus lineages co-circulating on both continents. In the past 10 years the European lineage viruses no longer appear to be circulating and there has been continued evolution of the American lineages with Florida Clade 1 and Clade 2 viruses dominating in Europe. The continued evolution of H3N8 viruses, albeit somewhat slower than that of human influenza viruses, is a challenge to vaccine manufacturers and the horse industry. In response to the major equine influenza outbreak in the UK in 1979, mandatory vaccination was introduced by the racing authorities in 1981 and a surveillance scheme was established with support from the Horserace Betting Levy Board and carried out by the Animal Health Trust. This remains the only coordinated endemic disease surveillance scheme in operation in the UK, and is both a reflection of the importance of equine influenza to the industry and a model for other infectious disease surveillance (Elton and Bryant 2011).

A viral cause of equine abortion was first identified by Dimock and Edwards in Kentucky in 1936, who showed that a filterable agent caused the disease. The virus, equine herpesvirus type 1, was characterised in Kentucky in the 1950s and, in response to catastrophic abortion storms in Kentucky studs, rapid progress was made with the development of diagnostic tests and the first vaccines to prevent these abortions (Doll 1961), which paved the way for the later periods of intensive study into equine herpesviruses from the 1990s to the present day. Contagious equine metritis was first identified in Newmarket in 1977 and, following a combined microbiological and epidemiological approach, diagnostic tests and an industry code of practice were quickly put in place, and control of the disease achieved.

Although this is not an extensive list, it gives some sense of how the understanding of equine infectious disease has progressed. It remains a highly challenging and dynamic discipline, especially with the continuing emergence of new equine infectious diseases. Hendra virus is one such example; first identified as a disease of flying foxes in Australia in 1996, the virus is endemic in Australia and has become a significant and serious zoonotic disease that has killed at least 80 horses and four people working in contact with horses, including equine practitioners (Mahalingam and others 2012). A Hendra virus vaccine launched at the end of 2012 is now being used to control the disease and so, 125 years on, equine infectious disease has returned to its One Health roots and the original work on glanders by Loeffler and Schuertz.

**New horizons in genomics and molecular biology**

The past 20 years have seen the power of molecular biology and genomics technologies applied to equine infectious diseases. *S. equi* was the first purely veterinary pathogen to have its complete genome sequenced (Holden and others 2009), a project undertaken by the Sanger Institute with financial support from the Horse Trust. Since then, veterinary microbial genome sequencing has become a widely used, rapid and economic scientific tool, with genomics forming the core of most current equine bacterial and viral research projects. Genomics has allowed the identification of the genetic machinery of microbes, providing previously unimaginable insights in evolution and ecology. New diagnostic tests have resulted from these approaches, which have greatly improved clinical practice and disease control in the field. Molecular diagnostic tests for the detection of microbial nucleic acid are now routinely used in equine diagnostic laboratories for a range of common bacterial and viral diseases including *S. equi*, equine herpesviruses and equine influenza virus. While conventional PCR assays remain highly important molecular diagnostic techniques, quantitative or real time PCR is a particularly powerful innovation because it provides an estimate of how much nucleic acid is present in the sample and hence an indication of the number of bacteria or virus genomes likely to be present in the sample. This highly sensitive technique has become a frontline diagnostic tool for the control of equine influenza and equine strangles, and allows differentiation of horses with acute equine herpesvirus infections from those with latent infections. New methods have become available for the genetic typing of bacteria. One of these, multilocus sequence typing, based on the sequences of conserved ‘housekeeping’ genes, has proven particularly powerful for comparing isolates based on their sequence type and determining relatedness between isolates. Application of this technique to *S. equi* and *Streptococcus zooepidemicus* has provided new insight into the genetic and phenotypic spectrum of these related bacteria and new understanding of pathogenicity (Webb and others 2008). We can expect to see further applications of molecular diagnostic techniques to equine pathogens and can look forward to an increasing number of horse-side diagnostic tests for specific viral and bacterial pathogens, as well as multiplex assays able to screen for a range of pathogens in a single test.

**Biofilms: complex bacterial communities**

Perhaps one of the major changes to have occurred in our understanding of microbial ecology is the understanding that bacteria behave differently in the host than in the laboratory. In the past 20 years there has been a sea change in the way we think of bacteria in the host, with the realisation that bacteria exist as complex, microbial communities called biofilms on the epithelial surface. In the past 125 years we have been on a journey from Koch’s reductionist microbiology, in which bacteria were studied as single cell organisms, to a view of bacteria existing in random, unstructured aggregates in a slime matrix in the 1980s and 1990s, to recognition of the biofilm in the past two decades. Biofilms fundamentally change the way we think about bacterial biology. Biofilms are complex, multispecies aggregates of bacteria within a polysaccharide matrix. Bacteria within a biofilm communicate via autoinducers in a process referred to as quorum sensing and are responsive to changes in the host environment (Rickard and others 2006). Importantly, biofilms show phenotypic variation, genome plasticity, increased antimicrobial resistance and increased ability to evade host defenses. As the biology of equine microfilms becomes better understood, it is likely that clinical
practice will change to take into account the impact of biofilms on the interpretation of culture results – because not all bacteria in a biofilm are culturable – and on antimicrobial sensitivity, since resistance patterns may be different in the host compared to the diagnostic laboratory.

Importance of surveillance

Looking ahead, however, some things will remain just as important as at present: equine practitioners and scientists need to be vigilant for changing patterns in equine infectious disease and the appearance of new variants of existing pathogens and of entirely new pathogens. Surveillance is central to identifying these trends and it will remain of critical importance that horses with suspected infectious disease, especially where the clinical picture is unusual, are investigated and that samples are taken for diagnostic testing.

It has been a fascinating 125 years that has taken microbiology and equine infectious diseases from their scientific birth with the likes of Pasteur, Koch and Schueter, through to the current genomic age. We can expect further innovation and change from both microbes and scientists over the next 125 years – a breathtaking struggle that impacts on all of us.

References


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