

Contagious bovine pleuropneumonia – never out of Africa?

Isaac D. Olorunshola¹, Andrew R. Peters², Massimo Scacchia³ and Robin A. J. Nicholas^{4*}

Address: ¹Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ilorin, Nigeria. ²Supporting Evidence Based Interventions (SEBI), Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, EH25 9RG, UK.

³Head Animal Health Laboratory Expert at OIE Reference Laboratory for CBPP, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale," Via Campo Boario, 64100 Teramo, ITALY. ⁴The Oaks, Nutshell Lane, Upper Hale, Farnham, Surrey GU9 0HG, UK.

***Correspondence:** Robin A. J. Nicholas. Email: robin.nicholas@btinternet.com

Received: 22 March 2017

Accepted: 8 May 2017

doi: 10.1079/PAVSNNR201712019

The electronic version of this article is the definitive one. It is located here: <http://www.cabi.org/cabreviews>

© CAB International 2017 (Online ISSN 1749-8848)

Abstract

Despite being eradicated from Europe and probably the rest of the world, contagious bovine pleuropneumonia (CBPP) continues to plague sub-Saharan Africa affecting at least 25 countries, in particular Ethiopia, Ghana, Tanzania, Cameroon, Angola, Nigeria and Zambia. Evidence has shown that the causative agent, *Mycoplasma mycoides* subsp. *mycoides*, may have emerged from a closely related goat pathogen as recently as 300 years ago, which corresponds to the first description of CBPP in Europe. Numerous new vaccines have been produced over the last 20 years in an effort to improve on the protection afforded by the T1/44, a live vaccine in continuous use in Africa for over 60 years but none have so far succeeded; indeed, many have exacerbated the condition. In this review, we argue that the present tools for diagnosis and control are adequate for eradication and what is necessary are resources to improve vaccine coverage to levels of at least 80%, last seen in the 1970s when CBPP was restricted to very few countries of Africa. In addition, a study in Namibia supported the concept that strategic and targeted antimicrobial treatment can play a critical part in the control alongside regular and comprehensive vaccination.

Keywords: *Mycoplasma*, CBPP, Cattle, Vaccines, Chemotherapy

Review Methodology: The authors are all experts in the CBPP field, are familiar with the recent and historic literature and know the main researchers and their work; information for the review was obtained from their personal collections of relevant articles and supplemented by personal experience, current awareness services provided by their respective institutions and by regular use of PubMed and CAB abstracts.

Introduction

In 1993, the Ad Hoc OIE Committee on contagious bovine pleuropneumonia (CBPP) met in Paris to draw up plans to eradicate this centuries-old disease from all parts of the world. This initiative was stimulated by the alarming spread of CBPP in sub-Saharan Africa and its unexpected re-emergence in southern Europe in the 1980s after an apparent absence of over a decade. At one point, CBPP simultaneously affected France, Portugal, Spain and then spread to Italy causing significant economic impact [1]. The disease was eventually stamped out in Europe by test and slaughter with the last case seen in northern Portugal in 1999. In Africa, however, despite the great advances in diagnostic technology and improved epidemiological tools over the last 20 years, it is fair to say that eradication is as far

off as ever with the disease endemic in at least 25 countries. So what has gone wrong in Africa?

Part of the problem appears to be the obsessive focus on development on new vaccines and diagnostic tests when we would argue that the present tools are adequate for eradication. Indeed, the most effective tool for detecting CBPP in Italy during the early 1990s was abattoir surveillance enabling trace-back to infected herds [2]. The continued investment in vaccines and tests unfortunately diverts funds away from the real task of increasing vaccine coverage with, admittedly, the far from perfect T1/44 vaccine. However, it should be remembered that this vaccine was sufficient to bring about a huge reduction of cases in many parts of Africa in the 1970s. At this time, veterinary services were functioning effectively and annual vaccine coverage in some countries was close to the 80%,

which is the minimum considered, necessary to give some hope of eradication [3]. Today there are few countries or regions in Africa, which are approaching even half this vaccine coverage.

In the last half of the twentieth century, the only African country to eradicate CBPP was Botswana, which was achieved by slaughter of all cattle in the infected region [4]. This of course was motivated by their lucrative export of cattle meat to the European Union, which would have been severely compromised had the disease been allowed to become endemic. However, this eradication had huge economic and political implications for the country in the short term.

This review looks at selective aspects of the developments in our knowledge of CBPP, including the evolution of the causative mycoplasma, *Mycoplasma mycoides* subsp. *mycoides*, its worldwide distribution and methods of control. For more comprehensive reviews, readers should refer to Egwu *et al.* [5], Nicholas *et al.* [4] and Nicholas *et al.* [1].

Evolution of the Causative Mycoplasma of CBPP

M. m. mycoides is a member of the *M. mycoides* cluster, which consists of five important ruminant pathogens, which share immunological, genetic and biochemical features. The decision to omit the 'SC' (small colony) from the name was taken as recently as 2012, when it became clear that there were sufficient differences between SC and the other member of the subspecies previously referred to as LC (large colony) to merit separation; LC became incorporated into the subspecies *capri* with which it was largely indistinguishable biologically and clinically [6].

CBPP was thought by some sources to have existed 'since time immemorial' [7], although detailed descriptions do not appear until the late eighteenth century when Bourgelat first distinguished it from other lung diseases [8]. Evidence to date suggests that the origin of the disease may be relatively recent and possibly initiated by changes in livestock husbandry. The goat pathogen, *M. mycoides* subsp. *capri* shows great variation in the sequence of the 16S rDNA gene, frequently used as a taxonomic clock for bacteria, suggesting a long period of evolution [9]. *M. mycoides* subsp. *mycoides*, on the other hand, is remarkably homogenous with little sequence variation even between African and European strains. This could indicate a much more recent emergence. Furthermore, using next generation sequencing and Bayesian analysis, Dupuy *et al.* [10] were able to date the most recent common ancestor of *M. m. mycoides* to around the end of the seventeenth century (give or take 100 years) possibly evolving following infection of cattle by *M. m. capri*, as a result of mixed livestock farming. Interestingly, a similar event may have been seen with another member of the *M. mycoides* group: the genetically homogeneous *M. c. capripneumoniae*, the cause of contagious caprine

pleuropneumonia (CCPP); this mycoplasma may have mutated from the closely related but genetically diverse *M. c. capricolum*, although this is yet to be proven.

CBPP in Europe

After nearly 20 years absence of disease to date, it is probably safe to say that it is unlikely that CBPP will be seen in Europe again. There is no live trade of cattle from endemic areas of sub-Saharan Africa, and though European borders now stretch well into Asia, there is little evidence of pockets of CBPP existing there [1].

CBPP had persisted in Europe for the first half of the twentieth century before appearing to die out around 1967. Then something quite unexpected happened: the disease reappeared in France in 1980, Spain in 1981, Portugal in 1983 and finally in Italy in 1990. What was even more unusual was that isolates from these new outbreaks were almost identical. Furthermore, comparative analysis of the new European isolates with African strains showed that the former did not oxidise glycerol, resulting in little hydrogen peroxide production attributable to the lack of the enzyme L- α glycerol phosphate oxidase [11]; African strains on the other hand produced copious amounts of this toxic oxygen radical. Subsequently, genetic analysis revealed a genomic segment of 8.8 kb, present in African strains, which was completely missing in these new European strains [12]; within this region was a copy of the *lppB* gene, a potential virulence factor, and the glycerol uptake gene. A single European strain from outbreaks in France in 1967 before the re-emergence did not have this deletion. How had this deletion occurred in the European strains? While mycoplasmas are not averse to losing genes – a process that has been going on for over 2 billion years as they evolved degeneratively from cell-walled ancestors – the latest was a huge loss over a very short space of time.

Unfortunately, the events surrounding the deletion will probably remain a mystery, but concerns about CBPP in Europe were sufficiently grave that research into new vaccines was being carried out in some laboratories. This was not widely known as official policy to control CBPP in Europe centred on slaughter of affected herds because vaccine antibody would adversely affect serological disease surveillance. Did European laboratories have the technology at the time to attempt molecular attenuation or could serial passage, the most widely practised method, bring about such massive loss of genetic material? And most alarmingly, how could such a strain appear in cattle in four European countries in such a short space of time? It has been speculated that the movement of cattle 'immunised' with this so-called attenuated strain may have been responsible for these new outbreaks, but no evidence exists to support this [13].

An experimental infection in cattle in Switzerland, which compared a single new European strain with an African strain, provided some evidence to support the view that

this deletion produced strains that were less virulent than African ones [14]. But some doubts remained in those who saw the lesions in the unexpected outbreaks in Italy in the early 1990s and those in Portugal 1983–1999 because of the severity of the lesions, particularly early in the epidemic [1]. In fact mortality was reported in three French herds (up to 50% in one small herd) in the Pyrenees in 1980 [15]. While clinical signs were often absent because of the close confinement of the cattle and, of course, the widespread use of antibiotics in Europe, lung lesions were often spectacular, completely destroying whole lungs, although usually only one, a differential diagnostic characteristic of CBPP. Moreover, exercising these normally stationary animals could readily stimulate coughing. It was evident that as the disease progressed over time in Europe, it became more chronic in nature with the sequestrum becoming the dominant lesion, but this was also true in cattle in parts of Africa where CBPP was endemic. In addition, there was no evidence to suggest that strains in Europe before 1967 were particularly virulent. This cyclical nature of CBPP outbreaks was well known to researchers in Africa 40–50 years ago: as the epidemic progressed, the disease became milder [5]. Although a great deal of work has been done on the pathogenicity mechanisms of *M. m. mycoides*, there is still much unknown.

CBPP in Asia

In Asia, CBPP has been reported in recent times from India, Bangladesh and Myanmar, but these countries are now believed to be disease-free [16]. Little information in the English language emerged from China until the last decade. The disease caused great economic losses to China's cattle industry between the 1950s and 1970s severely affecting Mao's 'Great Leap Forward'. But, following an eradication campaign initially involving mass vaccination using a novel live vaccine, prepared initially in rabbits, with reported high immunogenicity and protection, followed by quarantine and slaughter, CBPP was eradicated in 1989 [17]. Sporadic outbreaks are believed to occur in the Middle East largely as a result of importation of cattle from North East Africa, though few reports have appeared lately.

Suspensions that CBPP was present in Turkey have not been confirmed by small surveys performed in the last few years. In the most comprehensive study, just under 1000 animals at abattoirs in eastern Turkey were tested by serological methods; clinical samples were also taken from cattle showing suspicious lung lesions [18]. Less than 0.5% of sera were seropositive and no *M. m. mycoides* was isolated from any lesions suggesting that CBPP was absent. The very low seroprevalence was consistent with false positivity rates of serological tests used.

There was little evidence also of the presence of CBPP in the majority of provinces in Afghanistan during a recent survey of cattle; the testing of sera by competitive ELISA and immunoblotting failed to show any positive sera.

However, the cattle tested represented only 0.03% of the total population of the country, so a larger survey is required to confirm these findings [19].

CBPP in Africa

It is well documented that CBPP was carried to South Africa in 1854 with an imported bull from the Netherlands, where it spread rapidly via trek oxen into the Transvaal where it killed 100 000 animals within 2 years. It further spread by cattle movements to countries now known as Namibia, Zimbabwe, Botswana and then eventually to Angola, Zambia and possibly to the Congo region. Some believe that the infection of North West and East Africa had separate origins possibly through Abyssinia (now Ethiopia) with a British expeditionary force led by General Napier in 1868 (R. Windsor personal communication). This is very unlikely as there was no evidence at all that India, from where the expedition started, was infected at the time; indeed, CBPP was not reported there until 1910. A third scenario suggests that CBPP was always present in Africa as accounts by a German explorer described 'lungenseuche' (the great cattle death) around Lake Chad in 1869, recognised possibly as CBPP, which was decimating cattle populations in the region; the explorer reported that these outbreaks were regular occurrences and appeared to have spread from the West [20]. However, this could also be explained by the colonization of West Africa by Portuguese settlers bringing infected cattle anytime between the sixteenth and nineteenth centuries as suggested by Dupuy *et al.* [10].

Evidence from molecular epidemiological studies, initially hampered by the close homogeneity of strains of *M. m. mycoides*, has now shed light on the diversity of strains throughout Africa. Using multilocus sequence analysis, Manso-Silvan *et al.* [21] divided African strains into three distinct groups: Central and Eastern African, West African and Southern African. Variable number tandem repeat analysis appeared more discriminatory and showed clear differences between strains from Southern and East Africa [22]. These studies could support a multi-origin scenario for CBPP into Africa but could equally be accounted for by the mutation of strains as they spread through Africa from a single source. Using next generation sequencing of several representative strains, Dupuy *et al.* [10] concluded that those circulating in sub-Saharan Africa today are descended from a strain that existed in the early nineteenth century, and given the margin of error of their methods, this is highly likely to be the introduction from the Netherlands midway through this century.

Data from the OIE show that up to 2015, at least 25 countries were affected by CBPP, although more are probably affected as some do not carry out regular or comprehensive surveillance. Countries reporting the most outbreaks in the decade up to 2015 include: Ethiopia (624), Ghana (585), Tanzania (173), Angola (168), Cameroon

(144), Zambia (127), Nigeria (123), Togo (123), Cote D'Ivoire (113), Bukina Faso (105), Benin (85), Congo (68), Chad (55), Uganda (51) and Kenya (51). It is interesting to note that the disease prevalence has hardly changed over the last 20 years showing that attempts at control have been mostly unsuccessful. The disease in sub-Saharan Africa is mostly characterized by a chronic endemicity with occasionally severe outbreaks when naïve herds are exposed to infected animals moved, often illegally, across borders. One such example involved cattle purchased in Zambia and brought across the Zambezi to the Caprivi region of Namibia, which led to huge losses of up to 50% mortality in some herds [23]. Namibia itself is mostly free of disease but suffers from incursions from Angola in areas close to the border ironically as a result of the improving military situation here leading to less well-manned security posts and broken fencing.

Control

Traditionally, CBPP in Africa has been controlled by the live T1/44 vaccine, which has been used for over 60 years. Vaccination has been shown to be successful when coverage of the cattle population of a country or region is high and maintained over successive years. However, it is well known that the vaccine provides only short-term immunity, can cause severe adverse reactions and is rarely effective in the face of an outbreak. Furthermore, mathematical modelling has shown that even a mass vaccination campaign over a 5-year period is unlikely to achieve eradication unless other strategies are used [24].

Previously, it was believed that mycoplasma infections did not respond well to antimicrobial treatment and indeed, their use was discouraged or prohibited because of the fear of creating carrier animals that could spread infection [8]. However, it is common knowledge that in the absence of an effective vaccine or sufficient coverage, farmers and field veterinarians will use antibiotics as the only available control option. In 2006, the first official use of antimicrobial treatment to control an outbreak of CBPP was reported in the Caprivi region of Namibia [25]. The disease had been introduced from Zambia in 2003 and outbreaks were very severe, with mortality as high as 50% in the small herds of the region. In total, there were 37 outbreaks, which killed many cattle. Vaccination was immediately implemented, but animals continued to die and there were serious concerns that the disease would become endemic and spread to other cattle-rearing areas of Namibia. It had previously been shown experimentally that treatment of all CBPP-affected cattle with the antimicrobial danofloxacin (Advocin; Pfizer Animal Health) could significantly reduce transmission of the mycoplasma to other cattle [25]. Therefore, all clinically affected or seropositive herds were treated in the Caprivi with the antimicrobial according to the manufacturer's recommendations [26]. The number of new cases fell immediately with only three

animals dying within the first 3 months after treatment and no more overt cases after that. Clinical monitoring continued for 2 years during which no more cases were reported. Annual vaccination was instigated following the last outbreak. In the years following antimicrobial treatment, there were only sporadic and minor outbreaks in the east of Caprivi, where it was not possible at the time of the outbreaks to treat all cattle because of the seasonal flooding of the Zambezi; importantly, there have been no cases in the main cattle-rearing region in the south-west of the Caprivi. The eradication of CBPP from this region, where some 100 000 cattle are kept, represents a significant achievement as it is known that CBPP can persist indefinitely in small populations [3].

Evidence had also been advanced that the effect of antimicrobial treatment is to accelerate the healing process by promoting fibrosis in the lung, further inhibiting mycoplasma transmission [1]. The results of the Caprivi study supported the concept that strategic and targeted antimicrobial treatment can play a critical part in the control of CBPP alongside regular and comprehensive vaccination. Mariner [3] has been an advocate of this approach and concluded: 'this is an example of innovation in civil society leading to the development of an evidence base for an institutional shift in policy'. The metaphylactic approach of antibiotic treatment is also the most effective way of preventing the development of antibiotic resistance (Peter Lees, personal communication).

In vitro antibiotic susceptibility testing of 41 isolates of the mycoplasma pathogen has shown that at least 10 antimicrobials were still effective against CBPP [27] and, despite their widespread use in Africa, antimicrobial resistance was not yet evident. Further work is necessary to check the current field situation and to identify and evaluate suitable, cost-effective antimicrobials, focusing on correct dosing regimens. Regular monitoring of antimicrobial resistance of field strains is, of course, another essential requirement.

Future Vaccines

For a new vaccine to replace T1/44, it should, ideally, be stable, given in a single dose, provide a longer duration of immunity and higher levels of protection and not cause adverse reactions. Few vaccines to date have met these criteria. It would seem that only live vaccines could achieve the majority of these targets such as the Ben-1 vaccine used in China, where protection rates with lyophilised product were reported to be 100% even after 2 years [17]. Trials to test this vaccine in the African environment are currently underway, though the unusual production method of generating enough pleural fluid in experimentally infected animals may provide a challenge.

A succession of inactivated vaccines has been tried and tested over the last two decades. Two new approaches to vaccination against CBPP were reported by Nicholas *et al.* [28]. The first consisted of a whole cell mycoplasma vaccine

inactivated with saponin (an approach, which had previously been found to be protective for bovine mycoplasmosis, contagious agalactia and CCPP) and the second comprised a recombinant subunit vaccine prepared from the highly immunogenic lipoprotein LppQ [14]. In spite of two vaccinations at six weekly intervals and high antibody responses, there was no evidence in the small number of animals used of any protection afforded by either preparation; indeed, there appeared to be an exacerbation of pathology in the vaccinated animals compared with unvaccinated contact controls. Lesions and fibrin were most extensive and pleural fluid more abundant in vaccinated animals. In the LppQ group, half the cattle died before the end of the experiment, while a quarter died in the saponin group. This compared to just under half that died in the control group.

Using more traditional approaches, Mwirigi *et al.* [29] compared the protective capacity of the live T1/44 vaccine with two inactivated preparations of the Afadé of *M. m. mycoides*, challenged with a virulent strain. The protection levels were poor for the formalin-inactivated vaccine, but the heat-inactivated preparation was marginally better than T1/44 at just over 80 and 74%, respectively. These findings indicate that low doses of heat-inactivated *M. m. mycoides* can offer protection to a level similar to the current live-attenuated (T1/44) vaccine formulation, though it would be more expensive to produce.

Antibody levels as detected by current serological tests have always been poor indicators of protection and indeed have frequently been linked to postchallenge pathological reactions. Schieck *et al.* [30] found that animals with CBPP sequestra had significantly higher antibody levels against surface proteins than the animals that cleared mycoplasma from their lungs. The authors suggested that high antibody titres might play a role in the establishment of pathological changes, such as vasculitis.

The N terminus of the transmembrane lipoprotein Q (LppQ-N') of *M. m. mycoides* has been identified as a major antigen and a possible virulence factor in CBPP. Mulongo *et al.* [31] immunized cattle with purified recombinant LppQ-N' formulated in Freund's adjuvant, which showed a strong seroconversion to the lipoprotein. However, the vaccine provoked severe post challenge glomerulonephritis probably brought about by the development of antigen-antibody immune complexes.

A study by Carozza *et al.* [32] showed the potential of a viral-vectored prototypic vaccine, which gave rapid and strong humoral and cell-mediated immune responses in mice against lipoprotein A, a major antigen of *M. m. mycoides*. This represented a first step in developing a recombinant vaccine against CBPP but is a long way from producing one for use in the field against CBPP. Further down the road towards a subunit vaccine is a Canadian-Kenyan consortium who have adopted a reverse vaccinology approach and identified groups of vaccine candidates that are recognized in naturally affected cattle [33]. Several cocktails of these antigens have been shown to be

protective in small groups of challenged cattle. It is hoped that the positive results can be extrapolated in larger trials with better protective rates and duration of immunity superior to the T1/44 vaccine.

Final Remarks

Despite frequent meetings at the Food and Agriculture Organization (FAO) in Rome on CBPP over the last 20 years including one titled: 'Antibiotics to the Rescue' in 2006 to discuss new approaches to control based on targeted use of chemotherapy, very little real activity is evident in the field, partly due to the lack of funds but more often as the result of the inability of the delegates to co-operate. There are reports that *in vivo* antibiotic trials and evaluation of the Chinese vaccine are beginning, but progress has been painfully slow. There are hopes that progress will accelerate now that the Bill and Melinda Gates Foundation have identified CBPP as a priority for funding. If international and national bodies are serious about eradicating or even controlling CBPP, then extra funds should be set aside to improve vaccine coverage using the T1/44 with targeted use of antibiotics in outbreaks overseen by international experts. The development and use of increasingly sensitive diagnostic and epidemiological tests will only confuse what is a relatively easy disease to identify and will divert funds to where it is really needed. Although slow in identifying the disease, the Italian veterinary control authorities eradicated the disease in less than 3 years by movement control, a policy of test and slaughter mainly based on abattoir surveillance and enabled by an effective animal identification system. This is presently not feasible amongst the numerous affected countries of sub-Saharan Africa. However, rinderpest was a much more difficult disease to eradicate but was eventually successfully cleared from the world. As outlined nearly 25 years ago in Paris, an eradication plan for CBPP is eminently achievable if the will and co-operation is there. For now, it seems that the elimination of CBPP is as far away now as it was then.

Acknowledgements

We are grateful to the following colleagues for their help with this review: Ortwin Aschenborn, Kevin Godinho, Roger Ayling and Georgina Tjipura-Zaire. This review is dedicated to our friend and colleague, Otto Hübschle (1945–2008).

References

1. Nicholas RAJ, Ayling RD, McAuliffe L. Contagious bovine pleuropneumonia. In *Mycoplasma Diseases of Ruminants*. Wallingford, UK, CABI; 2008, p. 69–97.
2. Regalla J, Caporale V, Giovannini A, Santini F, Martel JL, Penha Goncalves A. Manifestation and epidemiology of contagious bovine pleuropneumonia in Europe. *Revue*

- Scientifique et Technique (Office International of Epizootics) 1996;15:1309–29.
3. Mariner JC. More appropriate disease control policies for the developing world. Onderstepoort, Journal of Veterinary Research 2009;76:135–40.
 4. Nicholas RAJ, Bashiruddin JB, Ayling RD, Miles RJ. Contagious bovine pleuropneumonia: a review of recent development. Veterinary Bulletin 2000;70:827–38.
 5. Egwu G, Nicholas RAJ, Ameh JA, Bashiruddin JB. Contagious bovine pleuropneumonia: an update. Veterinary Bulletin 1996;66:195–208.
 6. Shahram M, Nicholas RAJ, Wood A, Miles RJ, Kelly D. Kinetics of substrate oxidation and hydrogen peroxide production by *Mycoplasma mycoides* subsp. *mycoides* large colony (LC) type and *Mycoplasma mycoides* subsp. *capri*. Research in Veterinary Science 2009;87:364–66.
 7. Blancou J. Early methods of surveillance and control for contagious bovine pleuropneumonia. Revue Scientifique et Technique (International Office of Epizootics) 1996;15:1263–82.
 8. Provost A, Perreau P, Bréard A, Le Goff C, Martel JL, Cottew GS. Contagious bovine pleuropneumonia. Revue Scientifique et Technique (International Office of Epizootics) 1987;6:625–79.
 9. Petersson B, Leitner T, Ronaghi M, Bölske G, Uhlen M, Johansson KE. Phylogeny of the *Mycoplasma mycoides* cluster as determined by sequence analysis of the 16S rRNA genes from the two rRNA operons. Journal of Bacteriology 1996;178:4131–42.
 10. Dupuy V, Manso-Silván L, Barbe V, Thebault P, Dordet-Frisoni E, Citti C, *et al.* Evolutionary history of contagious bovine pleuropneumonia using next generation sequencing of *Mycoplasma mycoides* subsp. *mycoides* 'Small Colony' PLoS ONE 2012;7:e46821.
 11. Houshaymi B, Miles RJ, Nicholas RAJ. Oxidation of glycerol differentiates African from European isolates of *Mycoplasma mycoides* subsp. *mycoides* SC (small colony). Veterinary Record 1997;140:182–3.
 12. Cheng X, Nicolet J, Pourmarat F, Regalla J, Thiaucourt F, Frey J. Insertion element IS1296 in *Mycoplasma mycoides* subsp. *mycoides* small colony identifies a European clonal line distinct from African and Australian strains. Microbiology 1995;141:3221–28.
 13. Nicholas RAJ. Contagious bovine pleuropneumonia: in search of the origins and virulence of lung sickness. Microbiologist 2009;10:41–3.
 14. Abdo EM, Nicolet J, Miserez R, Goncalves R, Regalla J, Griot C, *et al.* Humoral and bronchial immune responses in cattle experimentally infected with *Mycoplasma mycoides* subspecies *mycoides* small colony type. Veterinary Microbiology 1998;59:109–23.
 15. ter Laak EA. Contagious bovine pleuropneumonia a review. Veterinary Quarterly 1992;14:104–10.
 16. Anon. World Organisation for Animal Health (OIE) Annual Report. OIE, Paris; 2016.
 17. Xin J, Li Y, Nicholas RAJ, Chen C, Liu Y, Zhang M, *et al.* History of the prevalence and control of CBPP in China. Veterinary Journal 2011;191:166–70.
 18. Çetinkaya B, Öngör H, Karahan M, Kalender H, Lorenzon S, Thiaucourt F. Abattoir-based survey of contagious bovine pleuropneumonia in cattle in Turkey. Veterinary Record 2003;152:254–58.
 19. Bahir W, Omar O, Rosales RS, Hlusek M, Ziyi G, Schauwers W, *et al.* The search for OIE-listed ruminant mycoplasmas in Afghanistan. BMC Veterinary Research 2007; doi: 10.1186/s12917-017-1067-7.
 20. Weiss H. Dying cattle: some remarks on the impact of cattle epizootics in the Central Sudan during the Nineteenth Century. African Economic History 1998;26:173–99.
 21. Manso-Silvan L, Perrier X, Thiaucourt F. Phylogeny of the *Mycoplasma mycoides* cluster based on analysis of five conserved protein-coding sequences and possible implications for the taxonomy of the group. International Journal of Systematic and Evolutionary Microbiology 2007;57:2247–58.
 22. McAuliffe L, Ayling RD, Nicholas RAJ. Identification and characterisation of variable-number tandem-repeat markers for the molecular epidemiological analysis of *Mycoplasma mycoides* subspecies *mycoides* SC. FEMS Microbiology Letters 2007;276:181–8.
 23. Hubschle O, Godinho K, Nicholas RAJ. Danofloxacin treatment of cattle affected by CBPP. Veterinary Record 2004;155:404.
 24. Mariner J, Catley A. The dynamics of CBPP endemism and development of effective control strategies. Proceedings of the third FAO-AU/ IBAR-IAEA Consultative Group Meeting on CBPP in Africa; 2003 November 12–14, Rome, Italy; 2003. p. 76–80.
 25. Hubschle OJB, Ayling RD, Godinho K, Lukhele O, Tjipura-Zaire G, Rowan T, Nicholas RAJ. Danofloxacin (AdvocinTM) reduces the spread of contagious bovine pleuropneumonia to healthy in-contact cattle. Research in Veterinary Science 2004;81:304–9.
 26. Nicholas RAJ, Aschenborn HKO, Ayling RD, Loria GR, Lukhele O, Tjipura-Zaire G, Godinho K, Hubschle OJB. Effect of Advocin on the elimination of CBPP from the Caprivi Region of Namibia. In: CBPP Control: antibiotics to the rescue. Proceedings of FAO-OIE-AU/IBAR-IAEA Consultative Group Meeting on CBPP in Africa. 2006. FAO, Rome, Italy; 2007. p. 33–40.
 27. Ayling RD, Bisgaard-Frantzen S, Godinho K, Nicholas RAJ. Assessing the *in vitro* effectiveness of antimicrobials against *Mycoplasma mycoides* subsp. *mycoides* small colony type to reduce infections of contagious bovine pleuropneumonia. Antimicrobial Agents and Chemotherapy 2005;49:5162–65.
 28. Nicholas RAJ, Tjipura-Zaire G, Scacchia M, Frey J, Hubschle OJB. An inactivated vaccine against CBPP exacerbates disease in cattle. Proceedings of FAO-OIE-AU/IBAR-IAEA Consultative Group Meeting on CBPP in Africa. 2004. November, Rome, Italy; 2005. p. 91–7.
 29. Mwirigi M, Nkando I, Aye R, Soi R, Ochanda H, Berberov E, *et al.* Experimental evaluation of inactivated and live attenuated vaccines against *Mycoplasma mycoides* subsp. *mycoides*. Veterinary Immunology and Immunopathology 2016;169:63–7.
 30. Schieck E, Liljander A, Hamsten C, Gicheru N, Scacchia M, Sacchini F, *et al.* High antibody titres against predicted mycoplasma surface proteins do not prevent sequestration in infected lung tissue in the course of experimental contagious bovine pleuropneumonia. Veterinary Microbiology 2016;172:285–93.

31. Mulongo M, Frey J, Smith K, Schnier C, Wesonga H, Naessens J, McKeever D. Vaccination of cattle with the N terminus of LppQ of *Mycoplasma mycoides* subsp. *mycoides* results in type III immune complex disease upon experimental infection. *Infection and Immunity* 2015;83:1992–2000.
32. Carozza M, Rodrigues V, Unterfinger Y, Galea S, Couplier M, Klonjowski B, *et al.* An adenoviral vector expressing lipoprotein A, a major antigen of *Mycoplasma mycoides* subspecies *mycoides* elicits robust immune responses in mice. *Vaccine* 2015;33:141–8.
33. Nkando I, Perez-Casal J, Mwirigi M, Prysliak T, Townsend H, Berberov E *et al.* Recombinant *Mycoplasma mycoides* proteins elicit protective immune responses against contagious bovine pleuropneumonia. *Veterinary Immunology and Immunopathology* 2016;171:103–14.