Developing attenuated vaccines to control mycoplasmoses

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Temperature-sensitive strains of *Mycoplasma gallisepticum*, *M. synoviae* and *M. hyopneumoniae*, created using chemical mutagenesis, have proven to be effective vaccines against the three major mycoplasmoses causing disease in poultry and pigs. The use of these vaccines in poultry has significantly reduced reliance on antimicrobial therapy, with a consequent reduction in usage of macrolides. Recent advances in development of methods for genetic manipulation of mycoplasmas has enhanced our capacity to identify virulence genes, offering the prospect of the development of novel, rationally attenuated vaccines in the future.

Mycoplasmas continue to be a significant cause of disease in animals throughout the world, typically causing chronic infection and disease at mucosal surfaces, although some species are invasive and cause systemic disease, particularly localising in joints, and one group of mycoplasmas are exclusively parasites of red blood cells. Historically, live virulent vaccines, administered subcutaneously, were used to control contagious bovine pleuropneumonia[1], and such a vaccine was a component of the successful program to eradicate this disease from Australia[2,3]. More recently, attenuated strains of the causative agent, *Mycoplasma mycoides* subspecies *mycoides* small colony type, generated by passage in eggs, have been used successfully in Africa, although these vaccines are still administered subcutaneously[4].

While inactivated vaccines have been used in an attempt to control several mycoplasmoses of animals, generally the greatest success has been achieved using live vaccines. Inactivated vaccines typically offer relatively limited protection, presumably because the systemic humoral immune response they stimulate has little efficacy at mucosal surfaces, and in some cases have resulted in exacerbation of disease after infection. Over the last 30 years there have been several notable successes in the development of effective, live, attenuated vaccines, suitable for administration into the respiratory tract, to control several of the most significant pathogenic mycoplasmas in farmed livestock, using an approach pioneered in the laboratory of Kevin Whithear at The University of Melbourne[5-10]. These successful vaccines have all been based on the derivation of temperature-sensitive strains from wild type strains by exposing them to a chemical mutagen, then selecting clones unable to grow at the core body temperature of the host. The first of these developed and commercialised was the ts-11 strain of *Mycoplasma gallisepticum*. This strain can infect the upper respiratory tract of chickens and persist for extended periods, but does not induce lower respiratory tract lesions and is not transmitted transovarially[9-11]. Extensive safety and efficacy studies clearly show that eye drop vaccination with ts-11 protects against disease induced by challenge with the wild type organism. Furthermore, although some vaccinated chickens do not develop detectable concentrations of serum antibody against *M. gallisepticum*, these birds are still protected against disease induced by challenge[12]. Recent studies have shown that the efficacy of this vaccine strain can be further enhanced by selecting clones of ts-11 that express the GapA adhesin[13].

Subsequently, chemical mutagenesis was used to create MS-H, a temperature-sensitive strain of *Mycoplasma synoviae*[8]. This strain was also shown to be safe and efficacious, when administered by eye drop, in preventing experimental infection in both chickens and turkeys, and also in preventing egg shell abnormalities in experimentally infected chickens[14-16]. Both these strains have now been commercialised in multiple countries throughout the world. Their use in Australia has greatly reduced the prevalence of mycoplasmosis in chickens and appears to have resulted in a tenfold reduction in the use of macrolides in poultry. Although selected as temperature-sensitive mutants, reisolates of both the ts-11 and MS-H strains that are no longer temperature-sensitive have been obtained from vaccinated flocks. However, these reisolates retain the attenuated phenotype, suggesting that the basis for attenuation is not temperature sensitivity, but some other, as yet unknown, mutation[17,18].

More recently, we have used the same approach to create an attenuated strain of the major porcine pathogen *Mycoplasma hyopneumoniae* and assessed it using a method for reproducing disease by exposure of piglets to an aerosol of cultures of *M. hyopneumoniae* in an enclosed chamber[19]. This strain, ts-19, does not induce lesions in pigs when they are experimentally infected and appears to induce protection in experimental studies when administered into the respiratory tract (unpublished data). While temperature-sensitive strains of the human pathogen *M. pneumoniae* were developed in the 1970s and preliminary studies suggested that they were attenuated and likely to be protective[20], some of the strains developed were insufficiently attenuated to be acceptable as vaccines, while others lost the capacity to colonise the oropharynx and did not stimulate an immune response. As a result, this approach to developing a human mycoplasma vaccine was discontinued, but possibly is worth revisiting given the subsequent success of the approach in domestic animals[21].

Understanding of the molecular pathogenesis of mycoplasmas has been limited by the lack of tools for genetic manipulation. Over the past 15 years there has been gradual development of some of the necessary tools, including plasmids utilising the chromosomal origin of replication[22], but targeted manipulation remains challenging and has not been achieved in some species, including *M. hyopneumoniae* and *M. synoviae*. These developments have enabled the identification of virulence genes in some mycoplasma species[23,24]. Directed mutagenesis of these virulence genes may offer a new approach to vaccine development in these species in the future. The capacity to manipulate the genome of mycoplasmas has also allowed the introduction of genes into vaccine strains. We have introduced chicken cytokine genes into the ts-11 strain of *M. gallisepticum* and have shown that this can overcome some of the immunosuppressive effects of this strain. We have also introduced and expressed a protective antigen from the viral respiratory pathogen infectious bronchitis virus[25,26]. These studies suggest that mycoplasmas may have potential as vaccine vectors, with their capacity to persist in their hosts, offering extended durations of vaccinal immunity.

In conclusion, there have been a number of significant advances in the development of vaccines to control mycoplasmoses in domestic animals. Improved control of these diseases not only has an impact on the health, welfare and productivity of domestic animals, but also has considerable implications for public health by decreasing the need for antimicrobial therapy in production animals.

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