Clostridial vaccines are commonly used in most countries where farming of cattle, sheep, goats and horses occurs on a commercial scale. Vaccines to protect against clostridial diseases make up the second largest group of ruminant vaccines sold globally. In Australia the sales value of these vaccines makes up $46m of the $96m sheep and cattle vaccine market (Baron market data). This group of vaccines has become so ubiquitous, and competition between competitors so fierce, that they have been reduced to the status of commodities where they can sell for less than 20c/dose.

However, this definition does not do justice to the enormous value they have generated for many decades ‘behind the farm gate’.

The clostridials grown for veterinary vaccines include some species important in human health viz: tetanus (C. tetani) and botulism (C. botulinum). C. perfringens D is of veterinary importance but it has a ‘close cousin’, i.e. C. perfringens type A, which was responsible for the deaths of so many soldiers in the trenches during WW1. However, added to those species of human importance are other species of mainly veterinary importance that can be found in vaccines sold in Australia, i.e. C. septicum, C. noyri Band C. chauvoei. Other species such as C. sordellii and C. baemolyticum and other members of the C. perfringens group complete the list of clostridials of global veterinary significance.

Members of the genus Clostridium are ubiquitous and are found in soil and in the gut of animals. Infection can be triggered by a variety of conditions ranging from a change in diet through to shearing cuts and dog bites. Toxins produced by the clostridia are responsible for much of the typical pathology. C. perfringens alpha-toxin is an enzyme, but this is the exception not the rule. Most of the other clostridial pore-forming toxins, such as C. septicum alpha-toxin and C. perfringens epsilon-toxin are not enzymes. C. perfringens alpha toxin is a phospholipase. Its basic enzyme effect is to ‘make a hole’ in the membrane of a cell by cleaving a cell membrane component. The cell contents leak out and the cell dies. The toxin of C. septicum acts in a similar way, forming a pore in the cell, again allowing the cell contents to leak. This ‘membrane-action’ seems to be a general trait of clostridial toxins. Although the specific signs of clostridial disease in animals depend on the causative species, gas formation and haemolysis are common. The progression of disease is usually rapid making treatment with antibiotics impractical. This is why vaccines are so vital. A review of the important clostridial toxins is found in Hatheway and a good coverage of the diseases of animals caused by the clostridia can be found in Hungerford.

All the clostridial vaccines sold in Australia are combination products. The combinations usually include up to five clostridial species, however different products also include leptospiral or Coryn bacterium pseudotuberculosis antigens. Two products made by Virbac combine a six-way vaccine with an anthelmintic (deworming chemical) to give sheep producers the added convenience of vaccinating and deworming in one shot. In South America some cattle vaccines contain up to 11 different antigens. Traditionally sheep and cattle are vaccinated twice in the first year of life and then receive annual boosters.

Production of clostridial vaccines involves growing the organism in fermenters, configured for anaerobes, to produce large amounts of toxin. Scale-up starts with a ‘seed’ vial taken from liquid nitrogen and grown up through several stages to the terminal fermenter, which for Virbac has a capacity of 6000 L. Each species usually has a specific media and set of fermentation conditions. This ‘know-how’ has been developed in-house over many years to give good toxin yields. However, despite high levels of quality compliance and good manufacturing practice (GMP) yields of toxin from fermentation batches still vary. This yield variability is common to our industry but is a frustration especially to the planners and the accountants. After toxin production has peaked, formalin is added to start the detoxification and inactivation process, turning toxins into toxoids. The formalin also kills the live bacteria. Following centrifugation to remove whole cells, and diafiltration across an ultrafilter, the concentrated ‘antigen’ is then stored, waiting to be blended into vaccines. For some antigens a whole cell culture is used in the final ‘blend’.

Antigens can be stored in a sterile concentrated stage for several years. Samples are taken at various stages throughout the
processing to measure toxoid yield and to confirm non-viability and non-toxicity.

Vaccines are blended based on registered inputs of toxoids which form part of the vaccine’s registration dossier. Adjuvants are added to non-specifically stimulate the immune response. At Virbac the adjuvants we use are aluminium salts or oil, depending on the duration of immunity required. Following blending, vaccines are aseptically filled, labelled and packed. Each batch of vaccine is tested for potency via antibody responses in rabbits and a battery of chemical tests is also conducted.

The basic technology used to make clostridial vaccines has not changed in many years. In fact the concept of using formalin to detoxify toxins produced by bacteria and the use of aluminium and oil as vaccine adjuvants, date back to the work of Ramon in the 1920s.

The veterinary vaccine world is highly regulated and products must be made and tested in compliance with the respective registration dossier and the rules and regulations of Good Manufacturing Practice. In Australia the veterinary industry is regulated by the Agricultural Pesticides and Veterinary Medicines Authority (APVMA) much in the same way the human medicine industry is regulated by the Therapeutic Goods Administration (TGA). Compliance, audits, standard operating procedures, specifications, change controls, validation and qualification are a necessary part of our industry that we live with day to day. This brings a high degree of rigour and control to those who work in this industry.

References


Biography

Dr Robert Dempster is the R&D and Licensing manager at Virbac Australia. He holds a MSc in microbiology and PhD in pathology. Virbac is a global animal health company. Robert has worked for eight local and international animal health companies in the past 28 years. He has held a number of technical and management roles but has spent most of his time working with vaccines for sheep and cattle.