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Role of live vaccines in antimicrobial stewardship in poultry

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Antibiotic use in Australian poultry production was commonplace during the rapid industry growth and intensification period from the mid- to late-1900s[1]. The range of antibiotic products available were readily applied prophylactically and therapeutically through feed, water and parenteral means for the control of clinical disease and to enhance growth. Antibiotics provided direct and indirect protection against a broad range of pathogens which underpinned flock health, productivity and facilitated industry expansion. Antimicrobial resistance was minimised by alternating medication programmes, potentiation through new combinations, and increasing dosage from prophylactic to therapeutic use. Antimicrobial stewardship was largely driven through the cost of production. However, by the 1980s, antibiotic resistance development in target and non-target organisms led the industry to look for alternate control strategies.

In the mid-to late 1900s vaccine development in Australia was largely confined to viral agents. Industry, public and private funds were channelled to develop a suite of live attenuated, controlled exposure, and inactivated products that were effective in controlling a range of respiratory and enteric pathogens. Bacterial agents were largely controlled by antibiotics, with a few autogenous inactivated vaccines. A cornerstone problem for the industry emerged with tylosin resistance in Mycoplasma gallisepticum[2]. Vertical transmission of tylosin-resistant M.gallisepticum (MG) strains from infected breeder flocks saw industry needing to find an effective solution to control respiratory disease in progeny flocks. Increased aerosolisation of MG in broiler and layer flocks increased the environmental challenge levels to breeder flocks creating an infection cycle in higher density growing areas. AgriFutures (formerly RIRDC) partnered with the University of Melbourne to develop a safe and effective live attenuated temperature-sensitive strain of MG (Vaxsafe® MG, strain ts-11) that colonised the upper respiratory tract of chickens stimulating long-term mucosal and systemic immunity[3, 4].Together with improved farm biosecurity, Vaxsafe MG has not only provided effective and sustained control of MG without the need for antibiotics, it has enabled the industry to essentially eradicate wild-type MG from Australian commercial poultry production, reduced selection pressure on antimicrobial resistance, and significantly reduced the amount of antibiotics used by the industry.

Relative to antibiotics, vaccines induce quite specific protection against pathogens. For overall flock productivity and welfare, health programmes must provide effective solutions spanning more than a single vaccine can provide, including the control of opportunistic or secondary pathogens, or the need for antibiotics may be reduced but not eliminated. Successful transition strategies to antibiotic-free farming have been delivered largely through a staged process. For example, anti-viral vaccines contribute to reduced antimicrobial use by preventing immunosuppression and thus secondary bacterial infections[5]. These can then be supported through targeting the development of vaccines against major pathogens, which in turn can then allow the use of narrower-spectrum antibiotics, or further reductions through the use of antibiotic shuttle programmes. Together, these measures form an antibiotic stewardship programme reducing antibiotic resistance pressure through an overall reduction and refinement in antibiotic use [6]. This was evidenced in the Australian poultry industry in the 1990s as Mycoplasma synoviae (MS) emerged as MG was bought under control through vaccination with Vaxsafe MG and antibiotic use was reduced. The industry again initiated a research programme to partner with the University of Melbourne, successfully developing a live attenuated temperature sensitive vaccine for the control of MS[7]. We now have a generation of chicken producers that has little experience with respiratory disease requiring antibiotic treatment owing to these two vaccines[8]. Equivalent live MG vaccines have been developed internationally[9] and demonstrated to be effective in eradication programmes[10]. However, these pathogens are not effectively controlled and remain problematic in many territories, particularly where lower biosecurity leads to higher challenge levels with persistent infections and antibiotic use remains. Indeed the emphasis on biosecurity has been elevated in antibiotic stewardship programmes such that if an agent can't be reliably excluded, a vaccine is probably needed[11]. Improving animal husbandry and nutrition has also been highlighted as an essential co-factor, especially incubation and brooding management due to their association with chick health and robustness[12].

Both live and inactivated vaccines have their strengths and weaknesses and are definitely not a 'silver bullet' for many conditions, particularly food safety commensal bacteria. When developing live vaccines it is ideal to have a detailed understanding of the pathogenesis of disease, develop a model for the desired mechanism of protection, and understand the relationship between optimal antigen delivery method and protection. General advantages of live vaccines compared to inactivated vaccines include i) broader antigen delivery through conformational and replication integrity preservation, ii) induction of mucosal and cell-mediated immunity in addition to humoral immunity, iii) lower administration cost through mass administration methods such as coarse-aerosol spray, drinking water and spray-on-feed, iv) they can be applied at a younger age such as in ovo or on the day of hatch, v) they have

a lower production cost (higher dose numbers per vial) and vi) favourable long-term storage and handling attributes as they can be lyophilised. Antigen preservation in the manufacture of live vaccines includes surface antigens such as glyco-, lipid-, somatic-, and structural-antigens (eg. flagellar and pilus). Live vaccines can also invade cells or be phagocytosed triggering cytotoxic and T-helper responses (CMI) against nuclear and structural proteins that more closely mimic natural infection. Thus, the antigenic and replication diversity induces a broader immune response including humoral, local and cell-mediated immunity. This is particularly important against pathogens that have multiple serovars such as Pasteurella multocida[13]. Mucosal immunity is a key advantage that theoretically increases the challenge dose threshold required for infection of wild-type organisms, and forms the basis of eradication or prevention programmes. Through stimulating CMI, live vaccines are highly beneficial against intra-cellular pathogens such as Eimeria sp[14].

Inactivated vaccines are safe as they don't replicate, can be quicker and easier to develop, and their formulation with adjuvants induces a strong humoral response that can make them more effective than live vaccines in high challenge environments (eg. Multi-age sites). They can be used under permit in Australia and are more versatile as strains or servovars can be exchanged in response to field isolates quite quickly. Inactivated vaccines have limited ability to prevent colonisation on mucosal surfaces such as those found in respiratory, reproductive and gastro-intestinal tracts, which are the most common portals of entry for pathogens in chickens[15, 16]. As inactivated vaccines only induce systemic humoral immunity, they induce clinical benefits by controlling pathogens that cause systemic infections, and to a lesser extent, reducing but not eliminating mucosal pathogens, thus requiring development of an inflammatory response and epithelial permeabilization for humoral antibody 'leakage' before they can bind mucosal pathogens. Infection with wild-type organisms may induce secondary bacterial infections that require antibiotic use thus reducing but not necessarily eliminating antibiotic use. Salmonella control in poultry is a good example. While infection can be reduced with both live and killed vaccines[17], the limited antigen expression of inactivated vaccines has required formulation with more than 1 serovar to be effective[18, 19]. However, the benefits of mucosal immunity have been associated with long term vaccination with a live attenuated Salmonella typhimurium (ST) vaccine in Australian layer chickens where reduced levels of environmental ST contamination, and this has been associated with reduced ST incidences in humans[20, 21].

Maintaining gut health without antibiotics has been a particularly challenging area. Chemical agents and antibiotics have been used traditionally to control bacterial and protozoal infections, as well as enhance the feed conversion efficiency. Some pathogens are synergistic and thus a non-antibiotic solution to one agent may still require therapeutic treatments to others. A good example is the need for dual Eimeria (coccidiosis) and Clostridium perfringens (necrotic enteritis) control[22]. Live attenuated Eimeria vaccines have provided effective control of coccidiosis, however, gut health and productivity can be negatively impacted unless C.perfringens is effectively controlled through optimising dietary formulation and inclusion of pre- and pro-biotics[23]. In the United States, live Eimeria vaccines are not attenuated and are applied as a controlled exposure strategy. Removal of ionophores in vaccinated flocks, which also have a controlling effect on Clostridium, has been associated with reduced performance and welfare parameters raising concern with producer and veterinary confidence in raising chickens without antibiotics[24]. A positive outcome of live Eimeria vaccines in a holistic farming strategy has been the replacement and reduction of antibiotic-resistant organisms[25].

Other challenges to the use of live vaccines for pathogen control has been the differentiation of vaccinated from infected (DIVA) flocks. Strain-specific assays have been required for users, technical advisors and the regulators. Live vaccine strain identification has co-evolved with the expansion of whole genome sequencing and high-throughput nucleic acid-based diagnostic assays such as quantitative PCR. DIVA assays are not only important to monitor vaccine uptake in flocks, but they also form part of a flock surveillance programme to ensure freedom of wild-type pathogen infections within the vaccinated population[26].

Antibiotics are still required by veterinary practitioners to address emerging diseases, or existing pathogens that re-emerge with changes to farming practices. For example, the shift of farming from intensive (caged) production to free-range allowed greater contact of chickens with environmental pathogens as well as their own when reared and grown on the ground (as opposed to cages). Campylobacter hepaticus causes Spotty Liver Disease emerged commercial layer and broiler breeder chickens[27, 28], and Pasteurella multocida that causes Fowl Cholera re-emerged due to higher challenge levels, difficulties associated with facility cleaning, and increasing serovar range. P.multocida is being largely controlled by fine tuning live and inactivated vaccine regimens, however, there are no effective vaccine solutions for C.hepaticus despite significant funding from industry levies over the past decade.

The future of live vaccine development is the targeted construction of recombinant vaccines. There are more recombinant-type vaccines used internationally today than conventional vaccines, especially for the control of viral diseases for example HVT- and Fowlpox-vectored vaccines for IBD, ILT and ND, and bacterial diseases such as Salmonella-vectored vaccines for necrotic enteritis[29]. These live recombinant vectored vaccines can be delivered

directly to a mucosal surface which not only allows for mass application but may also enhance mucosal immune responses[30]. Polyvalent and combination vaccines that target multiple pathogens may be more attractive and more efficient in reducing the need for antibiotics than monovalent vaccines[31].

While live vaccines have many advantages and are arguably the future of poultry health management, there are several major challenges to the speed with which they can be developed including a) the time required to undertake a full safety, efficacy and field testing clinical programme, b) substantial financial investment associated with the clinical studies programme, c) the inherent risk of achieving a saleable product and return on funds invested, and d) the additional regulatory burden associated with genetically-modified organisms, together with varying registration requirements in different territories. An intensive R&D preclinical studies programme required to assess the safety and efficacy of a vaccine can take a decade from concept to product launch. With greater understanding of immunology and pathogenesis of diseases our scientists are developing more targeted attenuation strategies. The availability of complete DNA sequences and a better understanding of gene function have allowed two specific strategies, either modifications or deletions to be introduced into the pathogen genome, with the aim of producing well-defined and stably attenuated live or inactivated vaccines[29], or tailored construction of vectored vaccines to express antigens of multiple pathogens. The development of these targeted vaccine constructs leads to safer and more efficacious vaccines, however it attracts considerable additional regulatory requirements for the handling, application and licensing of the vaccine under Australian GMO regulatory systems. Steps to reduce the regulatory burden are on foot, however the current and proposed systems will continue to drive investment into new solutions off-shore where the containment standards are more in line with a risk-based approach. Without non-antibiotic solutions for even smaller pathogens of livestock, there will be ongoing pressure to use antibiotics, or a delayed reduction.

In summary, the Australian poultry industry has seen a substantial shift over the last 30 years in flock health management. The industry is operating antimicrobial stewardship best practice and marketing products produced without antibiotic use whole of life. New challenges for the industry are based upon marketing and welfare drivers to change bird housing to free-range, and new solutions will need to be molecular-based requiring close collaboration between researchers, vaccine manufacturers and the regulators. The combined use of these safe and effective live vaccines, together with the available suite of live viral respiratory vaccines, improved biosecurity and animal husbandry, have allowed the Australian poultry industry to reduce secondary bacterial infections, reduce antibiotic use, and formed a critical part of the antibiotic stewardship programmes broadly adopted by the industry.

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