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Research review paper

The case for plant-made veterinary immunotherapeutics☆

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A B S T R A C T

The excessive use of antibiotics in food animal production has contributed to resistance in pathogenic bacteria, thereby triggering regulations and consumer demands to limit their use. Alternatives for disease control are therefore required that are cost-effective and compatible with intensive production. While vaccines are widely used and effective, they are available against a minority of animal diseases, and development of novel vaccines and other immunotherapeutics is therefore needed. Production of such proteins recombinantly in plants can provide products that are effective and safe, can be orally administered with minimal processing, and are easily scalable with a relatively low capital investment. The present report thus advocates the use of plants for producing vaccines and antibodies to protect farm animals from diseases that have thus far been managed with antibiotics; and highlights recent advances in product efficacy, competitiveness, and regulatory approval.

Abbreviations: CT, cholera toxin; CTB, B subunit of CT; ELP, elastin-like polypeptide; ETEC, enterotoxigenic *Escherichia coli*; IgG, immunoglobulin G; IgA, immunoglobulin A; sIgA, secretory immunoglobulin A; LT, thermolabile enterotoxin; LTB, B subunit of LT; PRRSV, porcine respiratory and reproductive syndrome virus; PWD, postweaning diarrhea disease; VHH, single variable domain on a heavy chain; VLPs, Virus-like particles.

☆ The opinions expressed and arguments employed in this publication are the sole responsibility of the authors and do not necessarily reflect those of the OECD or of the governments of its Member countries.

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Contents

1. The looming challenges for food animal production

The development of resistance to virtually every clinically-important antibiotic currently available for the treatment of bacterial infection is an important global health challenge. In the worst case the end of the “antibiotic era” would greatly increase human mortality, morbidity and health care costs. The primary driver for antibiotic resistance is thought to be the improper or excessive use of antibiotics in human medicine and in food animal production. Recently, the World Health Organization, the UK government and the G8 governments have emphasized the need for judicious use of antibiotics in agriculture as a key element of strategies to prevent or delay the onset of antibiotic resistance (G8 Science Ministers Statement, 2013; UK Department of Health, 2013; World Health Organization, 2012). These initiatives, coupled with a growing public demand for animal-based food which is “produced without antibiotics”, will undoubtedly constrain the availability and routine practice of using antibiotics for growth promotion and prophylaxis in livestock, poultry and fish production. Within this context, it is imperative to devise cost-effective strategies for the intensive production of livestock and fish using fewer antibiotics. Increased use of vaccines and immunotherapeutic agents will be a cornerstone of these strategies.

2. The need for efficacious vaccines and immunotherapeutic agents

Animal diseases have both direct costs — the immediate impact on livestock populations and agriculture — and indirect costs, such as mitigation or control efforts, losses in trade and other revenues, and impacts on human health. Zoonotic diseases are estimated to cause 75% of new emerging human infections, thus leading to significant morbidity and mortality, and creating costs in labor markets due to reduced trade and control measures. Diseases without zoonotic potential also impact human welfare costs through instability and increases in the cost of food. For example, the most recent estimate made in 2007 by the World Organization for Animal Health (OIE) of the direct impact of avian flu alone is $43 billion annually, while indirect costs are expected to be around $1.5 trillion [(The World Organisation for Animal Health, 2007), and tables 24–25 therein].

A variety of interventions can be used to combat bacterial (and viral) disease in animals, each with its own advantages and disadvantages (Table 1). Of the available alternatives to antibiotics, vaccination is likely the most widely used and effective strategy. Vaccination against viruses can also contribute to lower therapeutic use of antibiotics by reducing the incidence of secondary infections (Glass-Kaastra et al., 2013). Yet, vaccines and immunotherapeutics are available for only a limited number of animal diseases; and while global sales of animal health products in 2013 were $23 billion, only $5 billion corresponded to veterinary vaccines (Dolcera, 2014; Health for Animals, 2014).

Among the important veterinary diseases where current vaccines are not effective is porcine reproductive and respiratory syndrome virus (PRRSV), one of the most economically significant swine diseases in the world. A serious consequence of PRRSV infection is the loss of alveolar macrophages and therefore the weakening of the respiratory tract defense system, allowing secondary bacterial superinfections. Bacterial pathogens such as *Mycoplasma hyopneumoniae* cause more severe disease when PRRSV is present, and for this reason PRRSV outbreaks are often treated with antibiotics (Glass-Kaastra et al., 2013). Therefore, development of effective vaccines against viruses can lead to a reduction in antibiotic use in livestock. Furthermore, vaccination or the use of targeted immunotherapeutic antibodies can contribute to the maintenance of animal health, and offer promise as a pre-slaughter treatment to reduce meat contamination with zoonotic pathogens.

To be competitive, veterinary vaccines need to have a number of desirable attributes, many of which are met using plant-based production (Table 2). Many candidate subunit vaccines have been produced in plants and tested in target animals with positive outcomes (Kolotilin et al., 2014). Table 3 lists platforms that have been used for veterinary subunit vaccine production and examples of successful trials. Key aspects of the advantages of plant-based versus other platforms are discussed in the following paragraphs.

3. Attributes of plant-made pharmaceutical proteins

Compared to other platforms, plant-based production of recombinant proteins offers enhanced safety, reduced capital investment in infrastructure, and easy scale-up (Floss et al., 2007; Stoger et al., 2014). In terms of safety, plants have the evolutionary advantage of not being host to any prions, viruses, bacteria, or mycoplasmas that are infective to animals or humans. Progress towards high yields and product quality has also been achieved through advances in fundamental knowledge of heterologous gene expression and development of robust expression methods such as the use of transient expression through agro-infiltration of binary or viral vectors (Salazar-Gonzalez et al., 2015; Vézina et al., 2009), chloroplast transformation (Jin and Daniell, 2015), subcellular targeting and the use of suppressors of post-transcriptional gene silencing (Alvarez et al., 2008; Alvarez et al., 2010). Plants also provide eukaryotic-type processing and post-translational modifications, and modified expression systems are being developed that provide functionally-improved therapeutic proteins especially in terms of N-glycosylation (Steinkellner and Castilho, 2015).

Numerous bacterial and viral antigens have been expressed in plants and tested with positive results in the target animal species (Table 3). Similar approaches have been employed for prototype vaccines for use in humans including influenza, hepatitis B, Norwalk virus, rotavirus, human papillomavirus, hepatitis C and others (Gomez et al., 2009; Hernandez et al., 2014; Landry et al., 2010; Thanavala et al., 2005; Yusibov et al., 2011). However, for human vaccines an absolute requirement is high product purity, which remains challenging with plant-based products, making veterinary vaccine production in plants more attractive (see Section 6).

While there are no studies comparing process economics in various production systems, the cost of unpurified therapeutic protein