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Review article

New trends in innovative vaccine development against *Actinobacillus pleuropneumoniae*

Abraham Loera-Muro[a], Carlos Angulo[b,*]

[a] CONACYT-Centro de Investigaciones Biológicas del Noroeste, SC, Instituto Politécnico Nacional 195, Playa Palo de Santa Rita Sur, La Paz, B.C.S., C.P. 23096, Mexico
[b] Grupo de Inmunología & Vacunología, Centro de Investigaciones Biológicas del Noroeste, SC, Av. Instituto Politécnico Nacional 195, Playa Palo de Santa Rita Sur, La Paz, B.C.S., C.P. 23096, Mexico

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ABSTRACT

Actinobacillus pleuropneumoniae is the etiological agent of porcine pleuropneumonia, a respiratory disease leading to severe economic losses in the swine industry. The most widely used commercial vaccines are bacterins comprising inactivated whole cells of *A. pleuropneumoniae* but these have only been partially effective in preventing disease. Innovative immuno-prophylactic preparations of *A. pleuropneumoniae* based on ApxI, ApxII, ApxIII, ApxIV toxins and outer membrane proteins, among others (i.e. RnhB, GalU, GalT, HflX, ComL, LolB, LppC), have high protective efficacy in mice and pigs. Some vaccine preparations have efficacy against homologous and heterologous *A. pleuropneumoniae* serovars, which constitute an important advance to control porcine pleuropneumonia. In this arena, subunit vaccines based on toxins are one of the most advanced and promising developments. Many research groups have focussed on the development of live attenuated vaccines comprising strains with inactivated Apx toxins and/or other virulence factors, their protective efficacy being determined in mouse and/or swine models. Other innovative approaches such as bacteria, yeast and plants as production and oral delivery platforms have been explored in animal models and the definitive host with encouraging results. In addition, further research into *A. pleuropneumoniae*-based DNA and nano-vaccines, as well as bioencapsulation of antigens in plants, is envisaged. Here, the recent findings and future trends in innovative vaccine development against *A. pleuropneumoniae* are reviewed and placed in perspective.

1. Introduction

Actinobacillus pleuropneumoniae is the causative agent of swine pleuropneumonia, a serious disease of pigs causing significant losses to the industry worldwide (Chiers et al., 2010). The pleuropneumonia disease is highly contagious. Hemorrhagic, fibrinous and necrotic lung lesions characterize swine pleuropneumonia, and the clinical features range from acute to chronic. Exposure to the organism may lead to chronic infection, such that animals fail to thrive; and even though they may survive, the pigs become asymptomatic carriers that transmit the disease to healthy herds. *Actinobacillus pleuropneumoniae* is a Gram-negative bacterium belonging to the *Pasteurellaceae* family (Chiers et al., 2010). Two *A. pleuropneumoniae* biotypes have been described based on their dependence of nicotinamide adenine dinucleotide (NAD) (Buettner et al., 2011). Likewise, sixteen *A. pleuropneumoniae* serovars have been classified worldwide (Bossé et al., 2017). Upon entry into the host, *A. pleuropneumoniae* adhere to epithelial tissue by specific surface cell receptors such as fibronectin (Hamer-Barrera et al., 2004). Transmission from infected to uninfected pigs can be direct or indirect (Tobias et al., 2013, 2014). In acute outbreaks, clinical signs and death can appear as soon as 24 h, and iron acquisition is critical for bacterial survival and disease development (Humann-Ziehank et al., 2014). *A. pleuropneumoniae* has several virulence factors that have been described so far, including lipopolysaccharide (LPS), exotoxins (Apx), capsule polysaccharide, proteases, type IV pilus, Flp pilus, autotransporters of adhesins and biofilm formation (Chiers et al., 2010; Tremblay et al., 2013). Among them, ApxI-III toxins are considered the major virulence factors. The immune responses against *A. pleuropneumoniae* include innate pro-inflammatory cytokine (IL-1beta, IL-6, IL-17, TNF-alpha) production during the first 12 hours post-infection (Wyns et al., 2015) where neutrophils and monocytes/macrophages are key involved cells (Ondrackova et al., 2013). In addition, acute phase proteins, defensin and lactoferrin may have a role in host defence against *A. pleuropneumoniae* (Kahlisch et al., 2009; Luna-Castro et al., 2014; Yang et al., 2015). Maternal antibody transfer is significant since bacterial exclusion in piglets can be achieved. In addition, specific antibodies are produced from seven days and keep circulating during months after infection, which contribute to the reduction of severity in clinical signs

* Corresponding author.
E-mail address: eangulo@cibnor.mx (C. Angulo).

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Table 1
Examples of commercially available vaccines against *A. pleuropneumoniae*.

Name.	Company.	Composition.
Aptovac®	Biowet PULAWY	Inactivated *App* serovar 2 and 6, and *P. multocida*.
Coglapix®	Ceva	Containing *App* inactivated with formaldehyde, preserved with merthiolate and adjuvanted with aluminium hydroxide gel, and toxins ApxI, ApxII and ApxIII.
Neumosuin®	HIPRA	Inactivated *App* serovar 2, 4 and 5.
Porcilis APP®	MSD Animal Health	Exotoxins (ApxI, ApxII, ApxIII) and a 42 kDa OMP.
Serkel PleuroAP®	VIPAZ	Inactivated *App* serovar 1, 2, 3, 4 and 5.
Suvaxyn Respifed APP®	Zoetis	Inactivated *App* serovar 1, 5 and 7.

App: *A. pleuropneumoniae*. Apx: *A. pleuropneumoniae* toxin. OMP: Outer Membrane Protein.

(Gardner et al., 1991; Bossé et al., 2002). Cell-mediated adaptive immunity has been scarcely investigated in infected pigs. Currently, it is only known that T cells CD8+ gamma-delta as well as Th17 cells (CD4 + CD8α^{dim}IL-17A+) are associated with immune responses against *A. pleuropneumoniae*-infected pigs (Faldyna et al., 2005; Sassu et al., 2017).

Antibiotics still represent the most effective measure for limiting mortality and clinical disease during *A. pleuropneumoniae* outbreaks in most parts of the world. Antibiotic therapy is used at the onset of symptoms to prevent mortality and the spread of infection (Archambault et al., 2012; Hathroubi et al., 2015). However, in recent years, some strains have begun to emerge with different levels of resistance to antibiotics (Bossé et al., 2015). Therefore, alternative immunotherapies have gained attention in the swine industry to fight against *A. pleuropneumoniae* infection. Among them, several successful vaccines against swine pleuropneumonia are on the market (Table 1). However, the currently available vaccines against *A. pleuropneumoniae,* have not been completely successful in preventing development of disease (Kruse et al., 2017). This review presents the current knowledge on the achievements in vaccination against *A. pleuropneumoniae* (Fig. 1). The first part will be focused on inactivated, live attenuated- and polysaccharide- based vaccines; and the second part will describe the recent developments on subunit-based vaccines being used with native, recombinant or DNA approaches. Discussion and perspectives are provided on possible candidates for the development of new vaccines to combat porcine pleuropneumonia.

2. Inactivated and live attenuated vaccines

2.1. Inactivated vaccines

At present, several inactivated cell-based vaccines deriving from *A. pleuropneumoniae* are used for pleuropneumonia prevention in many countries (Table 1). However, the most commercially available vaccines against *A. pleuropneumoniae* infection comprise inactivated whole-cell bacterins derived from various serovars of *A. pleuropneumoniae* (Lu et al., 2011; Lopez-Bermudez et al., 2014). Those “first-generation” vaccines were the first commercialized vaccines against *A. pleuropneumoniae* (Ramjeet et al., 2008). Nevertheless, bacterins confer partial protection against homologous serovars, but they do not usually protect against challenge with heterologous serovars or prevent colonization (Bossé et al., 2002). In several cases, whole-cell bacterins do not provide full protection against pleuropneumonia (Kim et al., 2010, 2016). Components of inactivated bacterins, as well as other types of vaccines, are not representative of those in vivo, e.g. lack key virulence factors, have been proposed as a reason for their lack of efficacy (Ramjeet et al., 2008).

Another approach is to use autologous vaccines that have been used against *A. pleuropneumoniae*, which are obtained by growing the pathogens from samples of infected swine in the farm and the subsequent pathogen inactivation (Van den Wyngaert et al., 2015). However, due to regulatory requirements and bio-safety issues, autologous vaccines are typically restricted to be used on the farm where *A. pleuropneumoniae* was isolated. Consequently, its major inconvenience is the impractical approach to generate a vaccine derived from an isolated strain at farm level. Ideally, a broadly effective vaccine worldwide is required, and efforts toward this goal are being pursued.

2.2. Live attenuated vaccines

Since capsular polysaccharides determine the serovar specificity of *A. pleuropneumoniae*, this feature has been exploited in the vaccinology

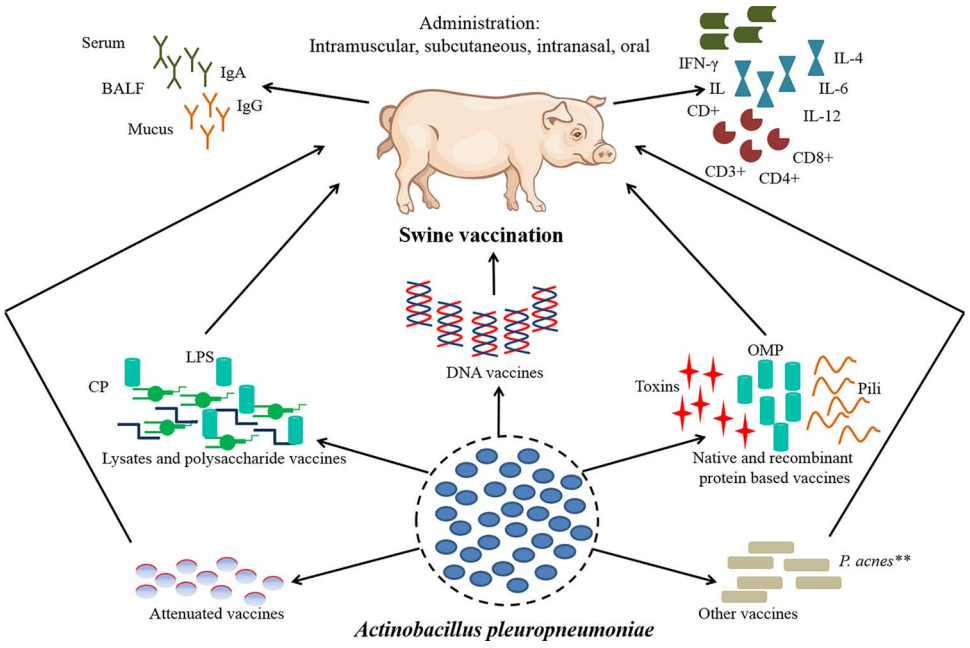

Fig. 1. Schematic representation of approaches, strategies and elicited immune responses implicated in *A. pleuropneumoniae* vaccine development. OMP: outer membrane proteins, LPS: lipopolysaccharide, CP: capsular polysaccharides, IL: interleukin, INF-γ: interferon gamma, CD+: cluster of differentiation, ***P. acnes*: *Propionibacterium acnes*, strategy used to develop a new vaccine against *A. pleuropneumoniae*.