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Efficacy of commercial swine influenza vaccines against challenge with a recent European H1N1 field isolate

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ABSTRACT

This study examines the immunogenicity and efficacy of four commercial swine influenza (SI) vaccines against challenge with a recent European H1N1 virus, Sw/Gent/112/07. The vaccines contained different H1N1 strains showing between 77% and 95% genetic homology with the haemagglutinin (HA) of the challenge virus. Four groups of 10 pigs each received a double vaccination, with a 4-week interval, with one of the vaccines; a fifth group served as unvaccinated controls. All pigs were challenged 3 weeks after the second vaccination intratracheally with $10^{5.0}$ EID_{50} of Sw/Gent/112/07. Sera were examined in haemagglutination inhibition (HI) tests against the homologous vaccine H1N1 strains, the challenge virus and a panel of five recent H1N1 isolates. Pigs were euthanized at 24 or 72 h post-challenge and virus titres were determined in right and left lung halves. Two vaccines, in which the H1N1 strains showed a genetic homology of 93% and 89% to Sw/Gent/112/07, significantly reduced virus replication. The vaccine containing an H1N1 strain with 95% homology to Sw/Gent/112/07, did not offer significant protection, neither did it induce the highest HI titres. In general, pigs with HI antibody titres $\geq$20 against Sw/Gent/112/07 were virologically protected against challenge. HI titres against other viruses, however, differed compared to the challenge virus and between viruses. Our data clearly show that the genetic homology with the challenge virus is not the ultimate predictor for SI vaccine performance. The true reason for the differences in vaccine potency remains obscure because other factors, such as the antigen dose and/or the adjuvant, also differed between the vaccines.

1. Introduction

Three subtypes of influenza viruses are circulating in pigs in Western Europe (Brown, 2000). H1N1 and H3N2 swine influenza viruses (SIVs) have been enzootic in major swine producing countries since the 1980s. H1N2 viruses have been introduced in European swine over the last decade (Brown et al., 1995; Van Reeth et al., 2000). Recent serological investigations in Belgium, Germany, Italy and Spain, suggest that all three SIV subtypes co-circulate within their swine populations, while H1N1 is the main SIV subtype found in Central and Eastern European countries such as Poland and the Czech Republic (Van Reeth et al., 2008). The predominant H1N1 SIVs in Europe have an entirely avian genome and were introduced from wild ducks to pigs in 1979 (Pensaert et al., 1981). They are thus characterized as "avian-like" H1N1 viruses and are antigenically and genetically distinct from "classical swine" H1N1 viruses, which remain predominant in North America and Asia (Olsen et al., 2006). European H1N2 SIVs also differ significantly from "avian-like" H1N1 viruses because their H1 haemagglutinin (HA) has been derived

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from a human H1N1 virus from the 1980s (Brown et al., 1998), and swine H1N1 and H1N2 viruses generally fail to cross-react in haemagglutination inhibition (HI) tests.

Vaccination is the most efficient mean to control SIV infections. At this moment four bivalent swine influenza vaccines are available in Europe. They contain an H1N1 and an H3N2 strain, and are unlikely to offer protection against infection with H1N2 SIVs (Van Reeth et al., 2003a). A new trivalent vaccine, which includes H1N1, H3N2 and H1N2 SIVs has been developed and will become available to the market in the near future (Dürrwald, 2007). Unlike human and equine influenza vaccines, SIV vaccine manufacturers are not obliged to regularly replace their vaccine strains in order to antigenically match the currently circulating viruses. Most commercial SIV vaccines contain different H1N1 and/or H3N2 virus strains, including older isolates from the 1970s or the 1980s or more recent viruses isolated after 2000. At the same time, the amount of antigen, which is another important determinant of vaccine efficacy, is measured by different methods and probably also differs between vaccines. Additionally, different adjuvants are added to the preparations in order to enhance their immunogenicity. Although vaccine manufacturers conduct potency and efficacy tests for the registration of their product, their performance has never been tested in a single comparative study.

Despite the fact that SIVs do not drift as much as human influenza viruses, some extent of antigenic and genetic drift has been recorded (Campitelli et al., 1997; de Jong et al., 2001,2007; Van Reeth et al., 2004). It is frequently asked, therefore, whether vaccines containing older virus strains should be updated. In this context, we had previously demonstrated that a commercial vaccine containing the A/New Jersey/8/76 H1N1 virus could protect efficiently against challenge with an antigenically and genetically different H1N1 field isolate from 1998, provided that the HI antibody titres to the challenge strain were high enough (≥160) (Van Reeth et al., 2001). However, no data has been published on the performance of commercial SIV vaccines against contemporary European H1N1 SIVs isolated over the last few years.

In this study, pigs were vaccinated with three of the four commercial SIV vaccines and a new trivalent vaccine, each containing different H1N1 strains, namely A/New Jersey/8/76, Sw/Netherlands/25/80, Sw/Belgium/230/92 and Sw/Haselunne/2617/03. The efficacy of these vaccines was compared against challenge with a recent H1N1 SIV field isolate, Sw/Gent/112/07. The immunogenicity against a panel of recently isolated H1N1 SIVs from different European countries was also investigated. Additionally, sequencing of the HA glycoprotein of the challenge virus was conducted and compared to that of the vaccine strains to investigate the possible correlation between genetic similarity and protection.

2. Materials and methods

2.1. Pigs

Forty-nine pigs from an influenza virus-seronegative farm were purchased for this study. The animals arrived at the experimental facilities at the age of 7 weeks, 1 week before the onset of the experiment. They were randomly divided in five groups as explained under experimental design. Each experimental group was housed in a separate isolation unit with HEPA filtered air. Food and water were provided ad libitum.

2.2. Vaccines

Four SIV vaccines were used in this study: Gripovac® (Merial SA, Lyon, France), Suvaxyn® Flu (Fort Dodge Animal Health, Naarden, The Netherlands), Respiporc® Flu (Impfstoffwerk Dessau-Tornau GmbH, Rodleben, Germany) and a trivalent vaccine which is not yet licensed and will be referred to as "Trivalent Flu". Table 1 shows the detailed content of each preparation according to the manufacturer's instructions note, including the vaccine strains, antigen doses and adjuvants used to enhance each vaccine's immunogenicity. The antigen dose is expressed in a different unit depending on the manufacturer and, therefore, cannot be directly compared.

Table 1
SIV vaccines used in the study and their detailed composition and percentage of amino acid (aa) identity between the HA1 segment of the haemagglutinin (HA) protein of vaccine H1N1 strains and the challenge virus.

Vaccine	Influenza virus strains	Production substrate	Type of vaccine	Adjuvant	Antigenic content per vaccine dose (2 ml)	% of aa identity with Sw/Gent/112/07
Gripovac®	New Jersey/8/76 (H1N1) Port Chalmers/1/73 (H3N2)	Eggs	Inactivated split vaccine	Oil-in-water emulsion	H1N1: ≥1.7 HIU H3N2: ≥2.2 HIU	77
Suvaxyn® Flu	Sw/Netherlands/25/80 (H1N1) Port Chalmers/1/73 (H3N2)	Eggs	Inactivated whole virus vaccine	Oil-in-water emulsion	4 μg HA of each subtype	89
Respiporc® Flu	Sw/Belgium/230/92 (H1N1) Sw/Belgium/220/92 (H3N2)	MDBK cells	Inactivated whole virus vaccine	Aluminium hydroxide-mineral oil	≥256 HAU of each subtype	93
Trivalent Flu	Sw/Haselunne/2617/03 (H1N1) Sw/Bakum/1769/03 (H3N2) Sw/Bakum/1832/00 (H1N2)	MDBK cells	Inactivated whole virus vaccine	Carbomer	$\geq 10^{7.0}$ $TCID_{50}$ of each subtype	95

MDBK: Madin-Darby bovine kidney; HIU: haemagglutination inhibiting units as determined by measuring the HI antibody response after the administration of the vaccine to pigs; HAU: haemagglutinating units before inactivation as determined in a haemagglutination assay with chicken red blood cells; $TCID_{50}$: tissue culture infectious dose 50% before inactivation.