Efficient protection against an experimental infection with heterologous strains of subtype H1N2 using a newly developed trivalent swine influenza virus vaccine

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Introduction and objectives

In the course of several reassortment events the novel swine influenza A virus subtype H1N2 emerged in the 1990s (1). This subtype had no reactivity in Hemagglutinition Inhibition (HI) tests with antisera of the classical swine H1 and avian-like swine H1 viruses (2) and was not covered by commercially available swine flu bivalent vaccines (3). Its lacking cross-reactivity to H1N1+H3N2 vaccines required the development of a new trivalent swine flu vaccine. Here we report the development of such a trivalent vaccine and the results of investigations of its efficacy against heterologous strains of the subtype H1N2 in experimental infection trials in pigs.

Materials and Methods

Three strains of subtypes H1N1, H3N2, and H1N2 were selected as vaccine strains (origin of H1N2: see Fig. 1). Viruses were grown in cell culture and inactivated. A substance from the carboxyvinylpolymers group was used as an adjuvant. Experimental batches were blended and investigated in laboratory and clinical trials (Fig. 2). A major focus of investigation was the proof of efficacy against heterologous H1N2 field strains in experimental infection trials. Five challenge experiments were conducted on a total of 156 pigs (see Table 1). Three recently isolated field isolates of subtype H1N2 were used for the experiments including the reassortant strain sw/Cloppenburg/IDT4777/2005 (see ref. 4). The strains were sequenced and analysed phylogenetically. Pigs were vaccinated twice and infected experimentally 7 days (TV0949/97), 4 months (TV 1060/06) or 6 months (TV 1059/06+1061/06) after second vaccination. Antibody kinetics after vaccination were investigated by HI. A very efficient challenge model was developed using the aerosol generator SAG-1 (5, see Fig. 3) that creates very fine droplets in a stable aerosol. SAG-1 consists of 2 containers, each of which can be filled with up to 1 L. There are 2 jets, one from each container, directed against each other. Spraying was performed in isolation rooms, with the generators suspended from the ceiling above the pigs, and all swine (up to 66 animals, see Tab. 1) were housed in one room. The containers of 3 generators were filled with medium from cell-cultured virus (1 L of medium with $10^{4.75}$ $TCID_{50}$/mL in trials 1050+1061/06, $10^{6.75}$ $TCID_{50}$/mL in trial 1060/06, $10^{7.25}$ $TCID_{50}$/mL in trial 0949/07a), the doors to the isolation rooms closed and an air pressure of 3-4 bar set on the generators. The generators produce droplets in the range 0.5 to 20 micrometers. Droplets in the range 5 to 20 μm are deposited in the trachea, bronchi and alveoli. Smaller droplets of 2 – 5 μm reach the alveoli and very small droplets of 0.2 – 0.5 μm are exhaled again. The ventilation system was switched off after starting of spraying so that the aerosol was constant for at least 2 hours. In one trial (0949/07, $10^{8.55}$ $TCID_{50}$ per pig) intratracheal infection was carried out in parallel in a separated infection unit because EP monograph 0963 requires an intratracheal infection model. Clinical scores were based on signs such as dyspnoea, fever, and body weight development.

Half of the animals (50% vaccinated and unvaccinated) from each trial were slaughtered on days 1 and 3 after challenge and necropsy performed. Tissue samples were taken from each lobe of the lungs, pooled, homogenized, diluted (log2) and injected into the allantois cavity of 11-day old embryonated chicken eggs to determine the virus titers over a period of days by hemagglutination. Standard histological (HE, PAS) and immunohistochemical investigations (SABC staining based on polyclonal rabbit sera against H1N2) were carried out with particular emphasis to investigate the degree of inflammation and to demonstrate the viruses in the lungs.

Trial	H1N2 Challenge strain FLUAV/sw/…	Challenge	Number of vaccinated pigs	Number of un-vaccinated pigs
1059/06	Groitzsch/IDT6016/2007	aerosol	14	14
1060/06	Cloppenburg/IDT4777/2005	aerosol	15	15
1061/06	Groitzsch/IDT6016/2007	aerosol	20	18
0949/07 a	Kitzen/IDT6142/2007	aerosol	15	15
0949/07 b	Kitzen/IDT6142/2007	intratracheal	15	15

Table 1: Overview of the experimental design

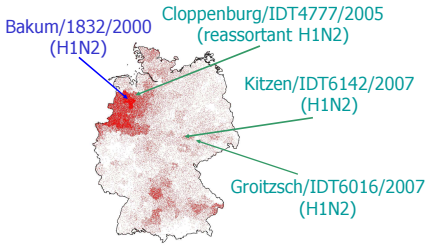

Fig. 1: Origin of H1N2 vaccine (1832/2000) and field strains; the red points reflect the density of the pig population (1 point corresponds to 500 pigs)

Fig. 3 a+b: Spraying of the infectious material

Fig. 2: Batch of the trivalent swine flu vaccine used for vaccination of the pigs in all 5 experiments

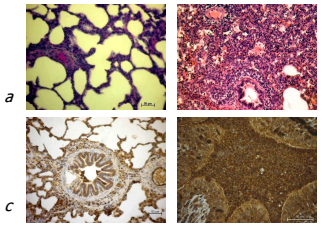

Fig. 5: Histological and immunohistological investigation, a HE vaccinated, b HE control, c SABC vaccinated, d SABC control

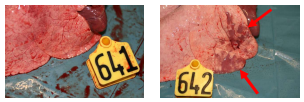

Fig. 4: Lung lesions (red arrows) 3 days after infection (trial 0949/07 a), a vaccinated, b unvaccinated

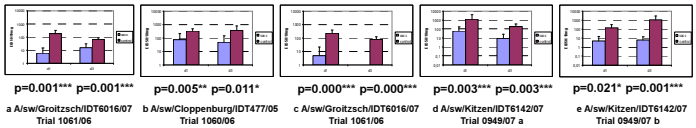

*Fig. 6 a-e: Viral lung load in egg infectious dose 50 (EID50) of vaccinated and nonvaccinated pigs after experimental infection with different H1N2 viruses; Statistics: Mann-Whitney U test (p= statistical probability, ≤ 0.005 significant *, ≤ 0.01 highly significant **, ≤ 0.005 very highly significant ***)*

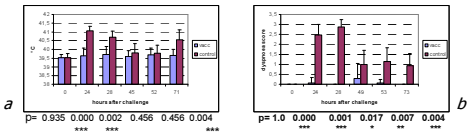

Fig. 7 : Evaluation of clinical parameters: a fever induction, b dyspnoea, data of trial 0949/07 a; Statistics: Mann-Whitney U test

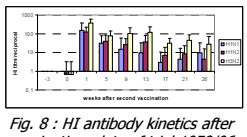

Fig. 8 : HI antibody kinetics after vaccination, data of trial 1059/06

Results

All pigs developed antibodies against the three subtypes contained in the vaccine. The antibodies were highest one week after second vaccination and decreased steadily thereafter (Fig. 8). The challenge infections induced the symptoms typical for swine influenza in the control groups: fever with two peaks and strong dyspnoea within the first three days after infection (Fig. 7). Vaccinated animals had almost no clinical symptoms after infection, whereas unvaccinated animals exhibited fever, dyspnoea and sleepiness. The viral load in the lungs was significantly lower in vaccinated pigs compared to unvaccinated animals (Fig. 6). Lung lesions were observed in the apical parts of medial and cranial lung lobes of the control animals, whereas vaccinated pigs showed no lesions (Fig. 4). Moreover, histological investigations revealed a higher degree of inflammation in the unvaccinated pigs (Fig. 5). The aerosol infection model was superior to intratracheal infection because it was more efficient, induced stronger clinical symptoms, imitated better natural infections and did not lead to artificial lung lesions as caused by intratracheal injection. Sequencing and phylogenetic analysis of the challenge strains demonstrated that they are heterologous to the vaccine strain (Fig. 9).

Discussion

Vaccinated pigs were protected against all three H1N2 field strains used in the challenge trials. Vaccinated pigs displayed a significantly lower viral lung load on day 1 and on day 3 after infection in all five experiments therewith fully complying with the EP monograph EP 0963 which requires significant lower viral lung loads in vaccinated pigs in comparison to control pigs after challenge with heterologous field strains. The heterologous character of the field strains was demonstrated by sequencing. The sequences differed from that of the vaccine strain. The field strains were 5 to 7 years younger than the vaccine strain. One field strain (Cloppenburg/IDT4777/05) reflected a newly emerged reassortant which contains the N2 of the H3N2 swine influenza A viruses (Fig. 9). This strain had shown limited neutralization by A/sw/Bakum/1832/00-like H1N2 viruses in *in vitro* experiments (4). Nevertheless protection against this strain was induced by the vaccine.

Thus a trivalent vaccine is available that protects efficiently against the newly emerged swine flu subtype H1N2. The vaccine is currently going through a centralised procedure of registration.

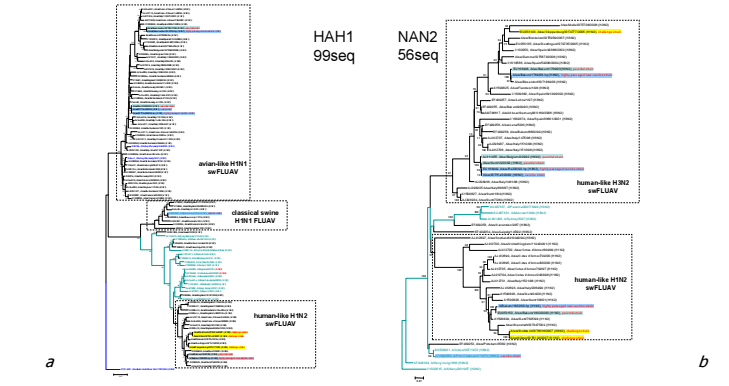

Fig. 9 : Phylogenetic analysis of vaccine and challenge strains; Neighbor Joining, Mega 4; 10000 bootstraps; Tamura Nei; a HAH1, b NAN2; blue inducated: strains from humans; blue background: vaccine strains; yellow background: challenge strains

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Acknowledgements

The authors thank Professor Dr. Jochen Süss, FLI, Jena, for providing influenza A virus strain/sw/Bakum/1832/00 (H1N2) and Dr. B.-A. Schwarz, LUA Sachsen, Leipzig, for providing isolates sw/Groitzsch/IDT6016/07 and sw/Kitzen/IDT6142/07 (H1N2) and Kerstin Wieczorek, Roswitha Ulrich, Katrin Schulz, Simone Köppen, and Dorit Appl, Research Laboratory Virology, R & D Department, IDT, for excellent technical assistence.

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