TECHNICAL REPORT

PAEROSOL efficacy toward airborne and surfaced H5N1 viruses.

MATERIALS AND METHODS

Introduction.

Influenza A is a pathogen of substantial public health concern worldwide. The evidence for human-to-human transmission of Influenza A H5N1 has identified it as a strain with pandemic potential, which would present significant challenges to the healthcare infrastructure in terms of both infection control and patient volume. H5N1 is easily transmissible between birds facilitating a potential global spread of H5N1. H5N1 is mainly spread by domestic poultry, both through the movements of infected birds and poultry products and through the use of infected poultry manure as fertilizer or feed. Humans with H5N1 have typically caught it from chickens, which were in turn infected by other poultry or waterfowl. Birds are also able to shed the virus for longer periods of time before their death, increasing the transmissibility of the virus. According to a report by the WHO, H5N1 may be spread indirectly. The report stated that the virus may sometimes stick to surfaces or get kicked up in fertilizer dust to infect people. H5N1 has mutated into a variety of strains with differing pathogenic profiles; some pathogenic to one species but not others, some pathogenic to multiple species. Each specific known genetic variation is traceable to a virus isolate of a specific case of infection. H5N1 has mutated into dozens of highly pathogenic varieties divided into genetic clades, which are known from specific isolates, but all currently belonging to genotype Z of avian influenza virus H5N1, now the dominant genotype.

There are the variety of chemicals reported effective for decontamination of avian flu virus including H5N1, however the most of decontaminating preps are chemically aggressive and not safe environmentally for humans and animals. Also, the proposed methodologies for the virus disinfection suggest the application of liquid decontaminants, which are not effective for decontamination of entire enclosed facilities, like food processing enterprises, chicken farms, fertilizers manufacturing facilities, etc.

The research was conducted to validate the effectiveness of PAEROSOL to simultaneously disinfect airborne and surface attached H5N1 virus. The research presents the results of ongoing study for PAEROSOL application for avian flu virus disinfection with possible application in poultry industry, including disinfecting of poultry farms, poultry processing facilities, poultry products and poultry manure.

1. Decontaminating agent

Electro-activated solution (EAS) was produced with a STEL unit (NPO "Ekran", Moscow) from solution of NaCl in distilled water and distilled water at a final concentration of NaCl - 1% and current intensity of 10 -11 A. The content of active chlorine in EAS - (0.10 ± 0.01) % and pH =7.0 ± 0.2. EAS was used fresh prepared without sterilization.

Herein, PAEROSOL is atomized EAS.

2. Viruses

Vaccine strain, Influenza virus A/NIBRG-14 (H5N1) was from the depository of the Research Institute of Influenza, RAMS (WHO Center for Influenza in RF). The virus was cultivated in the allantoic fluid of 10-12-day chick embryos for 48 hours at 36^0C, and the allantoic fluid was used for contamination. For the experiments on virus inactivation, the allantoic fluid containing $1x10^8$ EID_{50}/0.2 ml was used.

3. Titration of the virus

Virus titers in the control and the experimental samples were determined using the growing 10-12-day chicken embryos.

3.1. Virus samples were subjected to 10-fold serial dilutions in physiological solution ranging from 10^{-1} to 10^{-8}.

3.2. The prepared dilutions were injected with a syringe to allantoic cavities of chicken embryos (0.2 ml each), the opens sealed with paraffin, and the embryos were incubated in a thermostat for 48 hrs at 36^0 C. 2 embryos were used per each virus dilution.

3.3. Upon incubation termination, the embryos were cooled for a night at 4^0 C, the shells were opened up, and the allantoic fluid was sampled with a serological pipette. The sampled fluid was dispensed over the wells of a round-bottomed titration plate (0.1 ml/well), and an equal volume of 1% suspension of chicken erythrocytes in physiological solution was added to each well. The plates were incubated for 1 hr at a room temperature, and the virus infectivity titer was estimated by agglutination of erythrocytes in the wells.

3.4. Initial allantoic virus-containing fluid served as a control.

3.5. The value, inverse of a common logarithm of the original virus highest dilution, capable for induction of the positive hemagglutination reaction in a well, was assumed as the virus titer in control and experiment. The virus titer was expressed as the number of experimental infectivity doses (EID_{50}) or of common logarithms EID_{50} per a volume of the sample taken for titration.

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4. Virus inactivation

4. Virus inactivation on surface

4.1.1 0.1 ml of allantoic fluid infected with viruses was inoculated onto the surface of a sterile glass coupons as a coarse-dispersed particles generated by pneumatic atomizer (VMPS-MED, Fryazino, Moscow region)).

4.1.2 Contaminated coupons were dried at RT with a fan for 10 min, till no liquid was observed on the coupons. Then the coupons were positioned horizontally and vertically in 15 ft^3 - aerosol chamber.

4.1.3 Liquid EAS was atomized with VAG generator (one nozzle was operated) at the rate 10 ml/min to generate an aerosol with the drops size smaller than 10 μm and a drop mass median diameter d_{mmd} =3.6 μm (Table 1). A constantly operating fan was positioned in the chamber for uniform aerosol distribution.

4.1.4 The chamber with the coupons inside, remained tightly closed for additional 30 min after aerosol generation.

4.1.5 Then the coupons were withdrawn from the chamber. Samples were taken by washing virus down with sterile physiological solution, and the virus infectivity in each sample was determined by titration in chicken embryos as described in p.3 “Virus titration”.

4.1.6. The degree of influenza virus inactivation was evaluated by a decrease of the virus titer in the experiment compared to that in control.

4.1.7 In addition, samples were taken by washing virus down with sterile physiological solution from the coupons just after drying allantoic fluid on the glass coupons. Virus titers were controlled prior to each experiment.

4.1.8. Two coupons were used per one measured point.

4.2. Decontamination of airborne viruses

4.2.1. For the experiments on inactivation of an airborne virus, the virus-containing allantoic fluid was centrifuged at 4,000 rpm for 30 min at 4^0C. The obtained supernatant was centrifuged again at 20,000 rpm for 1 hr at 4^0C. The sediment was re-suspended in physiological solution to produce the virus suspension physically close to water and appropriate for fine-dispersed atomizing.

4.2.2. 1 ml of virus suspension containing 4 x 10^9 EID_{50}/ml was aerosolized (drops mass median diameter of 2.5 μm) by glass pneumatic atomizer positioned at the center of the chamber in order to achieve the virus concentration as 1.0 x 10^7 EID_{50}/m^3;

4.2.3. EAS was atomized with the VAG generator (one nozzle was operated) at the rate 10 ml/min (aerosol drops smaller than 10 μm and a drop mass median diameter =3.6 μm). A constantly operating fan positioned inside the chamber provided uniform aerosol distribution.

4.2.4. The control experiments were performed identically with physiological solution atomized instead of EAS.

4.2.5. *Experimental protocol*:

1. 1 min atomizing of virus-containing fluid inside the chamber
2. 0.5 min break for uniform distribution of virus particles through the chamber
3. 3 min sampling from chamber volume with micro-cyclone at the rate 7 L/min, (this is to estimate actual concentration of active viruses in the chamber prior to EAS atomizing)
4. 1 min atomizing of EAS (experiment) or physiological solution (control) with VAG generator.
5. 2 min break for uniform distribution of EAS (or physiological solution) aerosol in the chamber
6. 3 min sampling from the chamber volume with micro-cyclone at the rate 7 L/min (this is to estimate the concentration of the airborne virus in the chamber)
7. 2 min break
8. 3 min sampling from the chamber volume.

Air samples from the chamber were taken with 3 microcyclone devices positioned on the front and sides walls. According to the protocol, the contact time between airborne virus and PAEROSOL particles was measured as 4 and 9 minutes.

RESULTS AND DISCUSSION

PAEROSOL efficacy toward airborne and inoculated on glass coupons virus A/NIBRG (H5N1) is shown in Tables 1 and 2, correspondingly.

Table 1. PAEROSOL efficacy toward virus A/NIBRG-14 (H5N1) on glass surface as the function of atomized volume of PAEROSOL*

Experiment PAEROSOL atomized, ml /50ft^3		0	0.6	1.2	3.6	10.0
Influenza virus titer $log_{10}EID_{50}/cm^2$	1st exp	8.0 ±0.45	2.5±0.47	4.0±0.47	3.5±0.47	0
	2nd exp	7.8 ±0.47	4.0±0.47	5.0±0.47	3.0±0.47	0
Control Phys solution atomized, ml /50ft^3		0	0.6	1.2	3.6	10.0
Influenza virus titer $log_{10}EID_{50}/m^3$	1st exp	8.0±0.55	7.9±0.47	8.15±0.5	8.0±0.55	7.75±0.47
	2nd exp	7.8±0.47	8.2±0.4	7.85±0.5	7.8±0.	7.8±0.45

*Coupons remained in a contact with EAS aerosol for 30 min in airtight chamber

As seen from Table 1, positive correlation was observed between the volumes of atomized PAEROSOL and an efficacy of decontamination. Some scattering of the results could be explained by diffusion limitation associated with very low volume of disseminated PAEROSOL. As shown in Table 1, 10 ml of PAEROSOL distributed through the chamber of 50 ft^3 inactivated the virus on glass coupons below detection level during 30 minutes. Because of high safety requirements for experimenting with H5N1, the research was limited in variables. So that, it could be only stated that 3.6 ml of atomized PAEROSOL /50 ft^3 volume

was not enough to fully inactivate the viruses absorbed on glass surface, while 10 ml of atomized EAS disinfected the viruses below detection level. More experiments are required to research the optimal conditions, which allows for lesser PAEROSOL volume and/or shorter time of exposure.

To achieve high efficiency of PAEROSOL with minimal PAEROSOL volume is one of the ultimate goals of the project. So that, it could be proposed that increased contact time between viral particles and PAEROSOL could be used instead of increased volume of PAEROSOL applied. This assumption is supported by previously obtained data presented in the Report Task 2.2.4 (pp 7-8). There it was speculated that 2 factors contributed to the decontaminating effect of PAEROSOL - aerosol fraction and gaseous components.

Table 2. PAEROSOL efficacy toward airborne virus A/NIBRG-14 (H5N1)

	Virus infectivity titer (log_{10} EID_{50}/m^3.)		
	Time of the airborne virus contacting with physiological solution/EAS aerosol		
	0 min	4 min	9 min
Control 10 ml of MA of Phys solution 50ft^3	4.0 ±0.47	4.0 ±0.47	3.8 ±0.55
Experiment 10 ml of PAEROSOL/50 ft^3	4.33±0.5	0.0	0.0

As evident from Table 2, 10 ml of PAEROSOL distributed through the volume of 50 ft^3 disinfected airborne H5N1 virus during 4 min. Airborne virus infectivity was reduced below detection level in each of 3 conducted experiments.

The results presented above are in a full correlation with the results previously obtained with influenza virus A H1N1 in the frame of Task 2.2.3. Influenza A virus is essentially an avian virus that has "recently" crossed into mammals and therefore different strains of flu virus A are under precise attention. The results of Task 2.2.3 and the results of Task 2.9 provide very reliable evidence of PAEROSOL efficacy to decontaminate/disinfect airborne and surface adsorbed viruses.

CONCLUSION

- Decontaminating efficacy of atomized electrochemically activated solutions of 1% sodium chloride (PAEROSOL) was demonstrated with airborne and surface attached virus A/NIBRG-14 (H5N).
- PAEROSOL efficacy toward virus A/NIBRG-14 (H5N1)) fully correlated with PAEROSOL efficacy observed for A/Puerto Rico/8/34 (H1N1) virus