

The Effect of Some Physical and Chemical Agents on the Infectivity of the Highly Pathogenic Avian Influenza Virus in Egypt

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Abstract: In this study we have presented some aspects of the highly pathogenic avian influenza virus (HPIIV) in Egypt and the effect of some physical and chemical agents on its activity.

The effect of temperature and UV light on the infectivity of isolated avian influenza virus H5N1 virus in litter could be inactivated by increasing temperature above 50°C for at least 24 hr. UV light could not destroy the infectivity of the virus completely even after exposure for 48 hr.

Disinfectants evaluated in this study including Envirolyte, Virkon®-S 1%, Aldekol 0.5%, and bioscentry 0.5%. The results revealed that Envirolyte was very effective in reducing the titre of H5N1 virus after 10, 30 min and 12 hr of exposure at 25°C from (2^8) to (2^3) but it completely destroying the virus after 24 hr of exposure at 25°C with complete reduction in HA activity. The results also, revealed that Virkon-S® and Aldekol were effective in reducing the titre of virus after 30 min of exposure at 25°C to (2^3) without any additional reduction afterwards for Virkon-S®. While, Aldekol succeeded in reducing the titre of H5N1 virus after 12 hr of exposure at 25°C to (2^2) but without any inactivation of HA.

Keywords: HPAI; Physical agents; Disinfectants; Litter; HA.

Introduction

The highly pathogenic H5N1 viruses responsible for avian influenza outbreaks in Asia, and more recently Europe and Africa, (The recent unprecedented spread of highly pathogenic avian influenza "HPAI" across three continents) are considered mutants or reassortments of the first Asian H5N1 virus (Goose/GD/96) that was isolated from sick geese in southern China in 1996 [1] [2] [3].

HPAI is a disease of global concern because of the threat posed to food security in regions that are dependent on poultry as a main source of protein and livelihood. An additional concern is that the H5N1 virus may mutate and cause a human influenza pandemic in which millions of human lives would be threatened [4] [5] [3] [6].

In 2003, the highly pathogenic avian influenza (HPAI) H5N1 strain, starting circulating in Asia in 1996,

became enzootic in poultry. Indeed, from December 2003 to April 2005, HPAI H5N1 caused outbreaks of avian disease in nine Asian countries [7] [8]. This unprecedented spread of HPAI was associated with a failure of surveillance and control measures in these countries, allowing the spread of the virus to Middle East, then Europe in the summer of 2005, and later to Africa [9].

Egypt experienced the disease since the first introduction of highly pathogenic Avian Influenza HPAI H5N1 in 2006. The virus widely extended in very short time and infected commercial production sectors and backyards [10] [11].

Intensive poultry production systems in which a continuous and easily accessible source of susceptible hosts are considered prime conditions under which pathogenicity may emerge. Other cited conditions have been the inadequate use of vaccinations or

incomplete vaccination coverage that has allowed field strains to reassort with vaccinal strains [12].

In this study we have presented some ecological aspects of the isolated highly pathogenic avian influenza virus in Egypt and the effect of some physical and chemical agents on its activity.

Materials and Methods

Materials

Source of virus:

Egyptian H5N1 isolate. Influenza A virus (A/duck/Egypt/VRLCU-R28/2012(H5N1))

Litter:

Built up poultry litter was obtained from a commercial chicken farms in Giza Governorate.

PBS antibiotic solution:

Disinfectants:

-Vircon®-S

Composition: is powerful combination of various Potassium peroxymonosulfate, Sodium Chloride AND Other ingredients. Dilution Rate: 1.0% Solution

-Aldekol des® 03 (superior virucidal disinfectant)

Composition: IS powerful combination of various aldehydes with cationic biocides for disinfection.

Dilution Rate: 0.5%

-BioSentry® 904 Disinfectant

Active: combination of three quaternary ammonium components and TBTO Use: complete hatchery and farm disinfectant (0.5 %)

- Envirolyte-Anolyte (Env) It contains various mixed oxidants predominantly hypochlorous acid and sodium hypochlorite (HClO, ClO₂, HClO₃, HClO₄, H₂O₂,

O₂, ClO⁻, ClO₂⁻, ClO₃⁻, O⁻, HO₂⁻, OH⁻ - working substances, pH from 2.0 to 8.5, 1/500 =: 2 mg /L active chlorine, 1/1000=: 1mg /L active chlorine.

Methods

Preparation of H5N1 virus for contamination of litter

The preserved virus was cultivated in 9 to 11-day-old embryonated chicken eggs. Harvested amniotic fluid (AAF) was titrated on the basis of haemagglutination (HA) potential. Peptone water was prepared, autoclaved and incubated at 37°C for 24 h to check sterility. AAF was diluted in peptone water to have 4 HA unit titer. It was divided into aliquots in sterilized glass vials with 4 ml each. Each vial with H5N1 virus suspension was exposed to 4°C, 28°C and 56°C as well as ultraviolet light, for different time intervals [13].

Contamination of chicken litter [25]

Chicken litter from a commercial broiler farm was provided. The litter was packed in quantities of 300 g in autoclaved bags and incubated at 121 °C for 60 min. Moisture content of the litter were determined [14]. Three hundred grams of sterile chicken litter were poured under sterile conditions into sterile plastic boxes with lids. The chicken litter was contaminated with 4 ml of allantoic fluid of H5N1 diluted in 11 ml of sterile DMEM, which resulted in a titer of 10^{8.35} EID₅₀/300 g chicken litter.

The effect of temperature and UV light on the infectivity of avian influenza virus

Chicken litter was tested using an avian influenza test kit (Smart vet®) to ensure no contamination with AIV

occurred before the study. The manure samples were measured for moisture content [14] and pH [15] and

found to have 67% moisture content and a pH of 8.23.

The manure was dried in an incubator at 60°C for 72 hrs. Samples were divided into 4 groups as follows:

The first group was tested at 28°C. After each interval (1/2, 12, 24, 48 hrs) of exposure, one sample was taken out and added to sterile PBS to make a 20% suspension. The suspension was filtered with a 0.45 µm plastic filter (Minisart®, Sartorius). The filtrate was kept at -20°C until egg inoculation took place.

The second group was tested at 37°C.

The third group was tested at 56°C

The last group was subjected to UV light exposure at a density of 4-5 w/cm² (2⁵ °C), which was the average density during the day at the study site [16]. After each interval (1/2, 1, 1 1/2, 4, 12, 48 hr) of exposure, one sample was taken out and added to sterile PBS to make a 20% suspension. The suspension was filtered with a 0.45 µm plastic filter (Minisart®, Sartorius). The filtrate was kept at -20°C until egg inoculation took place.

Treatment with disinfectants

The solution of disinfectant was added to half of the boxes of contaminated chicken litter and half of the controls. The remaining boxes were left untreated with the compound. Once the disinfectant solution was added, the lid was closed and the chicken litter was manually mixed by rigorous shaking to distribute the compound equally through the litter. The boxes were incubated at room temperature (22 °C). Three samples of approximately 1 g each were obtained from three different randomly selected places in each box at 1, 12, 24, 36, and 48 hr post contamination by dipping a 50-ml reaction test tube into the litter under sterile conditions in a class 2 biosafety cabinet. The sample was resuspended in 5 ml of virus transport medium (13 minimal essential medium, 7.5% sodium bicarbonate, 15 mM 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES), 1% fetal bovine serum, 4000 U/ml penicillin, 400 mg/ml gentamycin, 8 mg/ml amphotericin B, 4000 mg/ml streptomycin, 1000 mg/ml kanamycin sulfate). The resuspended material was vortexed for 30 seconds and centrifuged at 3100 g for 20 min at 4 °C. The supernatant was aliquoted in three samples (1 ml each) and stored at 27° C until analysis.

Viability of the virus

The filtrate from each of the groups was inoculated into three 9-11 day-old chicken embryonated eggs according to WHO protocol [17]. After 48 hrs of incubation, the allantoic fluid was harvested and hemagglutination (HA) and hemagglutination inhibition (HI) tests were used to identify the recovered virus.

Table (1): selected group disinfectants with its manufacture recommended use dilution:

| Disinfectant company | Active ingredients | Recommended Use dilution | manufacture |
|--|--|--------------------------|---------------------------|
| Virkon- S [®] (20.4%) and NaCl (1.5%) | Potassium peroxymonosulfate | 1:120 | DuPont [™] (USA) |
| Aldekol Des- Gda [®] Chemkalien GmbH, | Activated Glutardialdehyde solution in combination with second generation quaternaries and non-ionic surfactants | 0.4% | EWABO |
| Biosentry [®] 904 [™] oxide 1% | QUACs 24% with tributyltin | 0.4% | DuPont [™] (USA) |
| Envirolyte-Anolyte It | contains various mixed oxidants | 1% | Germany |

Results and Discussion

Effect of physical methods on AI virus

Effect of temperature

Results of the physical inactivation of AI virus by temperature are recorded in Tables (2-a)

Table (2): Effect of temperature (28 ,37, 56°C) on activity of the avian influenza virus (The recovery of AIV).

| Exposure time (hours) | Exposure time (hours) | Embryonic death (%) | | | Exposure time (hours) | Embryonic dea | | Exposure time (hours) | Embryonic de | |
|-----------------------|-----------------------|---------------------|-------|-------|-----------------------|---------------|-------|-----------------------|--------------|-------|
| | | 28 °C | 37 °C | 56 °C | | 28 °C | 37 °C | | 28 °C | 37 °C |
| ½ | ½ | 100 | 100 | 100 | ½ | 100 | 100 | ½ | 100 | 100 |
| 12 | 12 | 100 | 100 | 100 | 12 | 100 | 100 | 12 | 100 | 100 |
| 24 | 24 | 100 | 100 | - | 24 | 100 | 100 | 24 | 100 | 100 |
| 48 | 48 | 100 | 100 | - | 48 | 100 | 100 | 48 | 100 | 100 |

Table (3): Effect of disinfectants on survival of the avian influenza Virus after 10 min exposure.

| Exposure time (hours) | Embryonic death (%) | | | HA Activity in the AAF | | | HI test | | |
|-----------------------|---------------------|-------|-------|------------------------|----------------|----------------|---------|-------|-------|
| | 28 °C | 37 °C | 56 °C | 28 °C | 37 °C | 56 °C | 2 8 °C | 37 °C | 56 °C |
| ½ | 100 | 100 | 100 | 2 ⁷ | 2 ⁷ | 2 ⁶ | +ve | +ve | +ve |
| 12 | 100 | 100 | 100 | 2 ⁶ | 2 ⁶ | 2 ⁶ | +ve | +ve | +ve |
| 24 | 100 | 100 | - | 2 ⁵ | 2 ⁵ | 0 | +ve | +ve | -ve |
| 48 | 100 | 100 | - | 2 ⁵ | 2 ⁴ | 0 | +ve | +ve | -ve |

From result we concluded that UV light could not destroy the infectivity of the virus completely even after exposure for 48 hr. This result agrees with Shahid *et al.*,

(2009)]18] who reported that UV light could not destroy the infectivity of the virus completely even after exposure for 4 hr. Distance from the source of light

and shallowness of the exposed suspension are also contributing factors in UV mediated viral destruction. Therefore, only microbes on the surface of material and in the air are killed by UV light. Similar results were obtained by [19] and [16]. UV light does not seem to be useful for viral destruction in fecal material. Although fecal material is not solid, it can still shield the virus from direct light. Therefore, only microbes on the surface of material and in the air are killed by UV light.

Effect of different commercial disinfectants on the survival of avian influenza virus H5N1 subtype

Disinfectants evaluated in this study including Envirolyte, Virkon®-S 1%, Aldekol 0.5%, and bioscentry 0.5%. From Tables (3) the results revealed that Envirolyte was very effective in reducing the titre of H5N1 virus after 10, 30 min and 12 hr of exposure at 25°C from (2^8) to (2^3) but after 24 hr of

exposure at 25°C completely destroying H5N1 virus with complete reduction in HA activity. The results also, revealed that Virkon- S® and Aldekol were effective in reducing the titre of H5N1 virus after 30 min of exposure at 25°C to (2^2) without any additional reduction afterwards for Virkon- S®. While, Aldekol succeeded in reducing the titre of H5N1 virus after 12 hr of exposure at 25°C to (2^2) but without any inactivation of HA. One reason for the reduction but not 100% inactivation might be explained with the fact that these compounds are used in a liquid form and they interact with the surface of the virus containing-matter and denature it [20] [21] [22].

There was complete reduction in the activity of HA in all exposure time to Envirolyte and Virkon- S®. Disinfectant induced inactivation of AIV has been reported by various researchers [23] [13] all over the world they used several chemical compounds and compound mixtures (acetic acid, citric acid, calcium hypochlorite, sodium hypochlorite, laundry detergent with peroxygen, commercial iodine/acid disinfectant) to disinfect LPAIV.

Muhammad *et al.* [13] reported the efficacy of Virkon-S against H7N3 subtype and found that 0.5% dilution was able to inactivate AIV fully after 90 min while 1% and 2% concentration achieved virucidal activity in just 30 min. They further described that phenol crystal at 0.2% and 0.4% dilution required 18 and 12 h respectively to kill the same virus which is contrary to present study findings where phenol crystal at 0.4% took only 15 min to kill H5N1 at 28°C. Ito *et al.* [24] has reported the effect of six povidone iodine products at 2, 0.5, 0.25, and 0.23% concentrations on HPAI A/crow/Kyoto/T2/04 (H5N1). The results showed virucidal activity at all concentrations reducing the virus infectious titers to levels below the detection limits of virus isolation only after 10 s at 25°C. It is not in agreement with our finding where Iodine crystals at 0.2% dilution were not able to inactivate H5N1

Virus even after 60 min but 0.4 and 0.6% inactivated after 15 min at 28°C. The inactivation of an enveloped virus is a process with components that bring lipids into solution, such as alcohol-containing disinfectants,

because of the nature of the envelope [25]. The use of chlorine inactivated HPAIV at a level of more than three orders of magnitude, but did not inactivate the virus completely.

From this study we indicate that the ambient conditions in the chicken litter were sufficient to eliminate the infectivity of the virus. This result was not surprising, as it is similar to results of [26]. The virus was also inactivated after 12 hr. without the addition of metam-sodium fumigant. This indicates that the ambient conditions in the chicken litter were sufficient to eliminate the infectivity of the virus. On the other hand, it has been noted that mixing of AIV with field chicken manure inactivated the virus 5–10 times faster than under unmixed conditions [27] These data and our data indicate that components in the chicken litter have a detrimental effect on the infectivity of AIV.

Conclusion

This study describes the effects of physical and chemical agents on infectivity of AIV H5N1. It is therefore inferred that H5N1 virus can be inactivated in the poultry farms/ hatcheries using high temperature (e.g. 56 °C), using of disinfectants of the material to be disinfected. However, it may not be practically feasible for the farmers. Use of disinfectants seems more appropriate and practicable. Consequently there is no need to depopulate the poultry sheds after AIV outbreak for long period of time before arrival of new stock if disinfectants are used appropriately. The disinfection of surfaces also plays a role in controlling the spread of disease to other facilities. Mostly liquid disinfectants have been used for this purpose.

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