Combating Avian Influenza H5N1 Virus with High Efficacy Disinfectant.

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Abstract—This article relates to high efficacy disinfectant that effectively combats avian flu influenza H5N1 Virus with 100% disinfecting efficacy.

Keywords- H5N1 Virus, High Efficacy Disinfectant, Influenza, Quaternary Ammonium Compound,

INTRODUCTION

The H5N1 highly pathogenic influenza viruses subtype have infected more than 600 people since 1997, resulting in the deaths of approximately 60% of those infected. [1]

The influenza A viruses circulating in avian species rarely infect humans. However, since 1997, highly pathogenic avian influenza viruses of the H5N1 subtype have infected more than 600 people. Infection of humans with these viruses typically leads to severe respiratory disease that often progresses to multiorgan failure; approximately 60% of confirmed cases of highly pathogenic H5N1 influenza infection have resulted in death.

The first fatal infections of humans with highly pathogenic avian H5N1 influenza viruses were reported in Hong Kong in 1997. [2]

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Since their emergence in the late 1990s, highly pathogenic avian H5N1 influenza viruses have undergone multiple reassortment events with avian influenza A viruses of different subtypes, including H6N1, H9N2 and H5N1 [3,4,5,6,7]. Hence, the currently circulating highly pathogenic H5N1 viruses represent a diverse group of viruses. Moreover, the viral surface glycoprotein HA (the major viral antigen), has evolved through point mutations, leading to a number of genetically and antigenically distinct clades and subclades.

The major clades circulating during the past years include clades circulating in Egypt, Israel, the Gaza strip and the West Bank, circulating in China, Bangladesh India and circulating in Indonesia. [8,9,10].

Although genetically and antigenically diverse, highly pathogenic avian H5N1 viruses share the ability to cause high mortality in poultry and infect humans. Recently, the HA gene of highly pathogenic avian H5N1 influenza viruses of clade 2.3.4.4 has reassorted with the neuraminidase (NA) and other viral genes originating from different avian influenza viruses, giving rise to novel viruses of the H5N2, H5N6 and H5N8 subtypes.

Many studies have assessed the virulence and pathogenicity of highly pathogenic avian H5N1 influenza viruses in different cell types and animal models including chickens, ducks, mice, guinea pigs, ferrets, pigs and nonhuman primates (reviewed in [11,12]). Mice are typically used to assess the virulence and immunogenicity of influenza viruses because they are inexpensive and multiple immunological reagents are available.

However, mice are not a natural host of influenza viruses and typically do not transmit viruses. Ferrets infected with influenza viruses show signs of respiratory infection similar to those observed in humans, and influenza viruses can transmit among ferrets via respiratory droplets.

This animal model is limited by its relatively high cost and the limited number of immunological reagents available. Guinea pigs have recently been established as an additional model for influenza virus transmission studies; however, infected animals typically do not show overt signs of disease. This review focuses on studies of the virulence and pathogenicity of highly pathogenic avian H5N1 influenza viruses in mammals. Despite of many attempts to eradicate these viruses by depredation of the live poultry markets in Hong Kong, H5N1 viruses re-appeared in 2001 and 2003 and are now enzootic in poultry in parts of Southeast Asia and the Middle East. Cases of infected wild and/or domestic birds have been reported by other Asian countries, as well as several European and African countries.

Most human infections with highly pathogenic avian H5N1 influenza viruses have occurred in Indonesia, Vietnam, Egypt, China and Cambodia. Egypt experienced a spike in human infections with these viruses in late 2014 and early 2015, but it is not clear whether this is because of more comprehensive screening and reporting, or because the highly pathogenic avian H5N1 influenza viruses circulating in Egypt have acquired mutations that facilitate human infection.

This article relates to the composition of a fast-acting disinfectant against the highly pathogenic H5N1 Virus by synergistically blending a major portion alcohol with minor portion of twin-chain quaternary ammonium compound, essential oil, non-ionic surfactant, and solvent. The use of synergistic combination of twin-chain quaternary ammonium compound, ethyl alcohol or methyl alcohol, non-ionic surfactant, essential oil and water as a fast-acting disinfectant to combat the current high lethality and virulence H5N1 Virus has been an area of considerable research and investigation of the said authors.

The article covers a new method of manufacture and a chemical composition that effectively combat H5N1 virus by synergistically combining twin-chain quaternary ammonium, alcohol, non-ionic surfactant, essential oil, and water.

It includes the use of anti-viral agent using Quaternary Ammonium Compound[13,14,15]-Dideycl Methyl Ammonium Chloride, Ethyl Alcohol [16] as a synergistic anti-viral and antimicrobial agent, Alcohol Ehtoxylate [17] as the cleaning and emulsifying agent, peppermint or spearmint [18,19,20] to further enhance the synergistic effect of the anti-viral properties, water [21]to enhance the solubility of above said chemicals and to create an exothermic reaction [21]with alcohol [21] to further enhance the solubility essential oil in said composition.

Avian influenza is an infection caused by avian (bird) influenza (flu) viruses. These influenza viruses occur naturally among birds. Wild birds worldwide carry the viruses in their intestines, but usually do not get sick from them. However, avian influenza is very contagious among birds and can make some domesticated birds, including chickens, ducks, and turkeys, very sick and kill them [22,23]

Infected birds shed influenza virus in their saliva, nasal [24] secretions, and feces. Susceptible birds become infected when they have contact with contaminated secretions or excretions or with surfaces that are contaminated with secretions or excretions from infected birds. Domesticated birds may become infected with avian influenza virus through direct contact with infected waterfowl or other infected poultry, or through contact with surfaces (such as dirt or cages) or materials (such as water or feed) that have been contaminated with the virus.

Usually, “avian influenza virus” refers to influenza A viruses found chiefly in birds, but infections with these viruses can occur in humans. The risk from avian influenza is generally low to most people, because the viruses do not usually infect humans.

However, confirmed cases of human infection from several subtypes of avian influenza infection have been reported since 1997. [25] Most cases of avian influenza infection in humans have resulted from contact with infected poultry (e.g., domesticated chicken, ducks, and turkeys) or surfaces contaminated with secretion/excretions from infected birds.

During an outbreak of avian influenza among poultry, there is a possible risk to people who have contact with infected birds or surfaces that have been contaminated with secretions or excretions from infected birds.

Of the human cases associated with the ongoing H5N1 outbreaks in poultry and wild birds in Asia and parts of Europe, the Near East and Africa, more than half of those people reported infected with the virus have died. Most cases have occurred in previously healthy

children and young adults and have resulted from direct or close contact with H5N1-infected poultry or H5N1-contaminated surfaces. In general, H5N1 remains a very rare disease in people. The H5N1 virus does not infect humans easily, and if a person is infected, it is very difficult for the virus to spread to another person.

Nonetheless, because all influenza viruses have the ability to change, scientists are concerned that H5N1 virus one day could be able to infect humans and spread easily from one person to another. Because these viruses do not commonly infect humans, there is little or no immune protection against them in the human population. If H5N1 virus were to gain the capacity to spread easily from person to person, an influenza pandemic (worldwide outbreak of disease) could begin.

No one can predict when a pandemic might occur. However, experts from around the world are watching the H5N1 situation in Asia and Europe very closely and are preparing for the possibility that the virus may begin to spread more easily and widely from person to person.
Due to the high lethality and virulence of H5N1 Virus, its endemic presence, its increasingly large host reservoir, and its significant ongoing mutations, the H5N1 virus is the world's largest current pandemic threat. The world is teetering on the
edge of a pandemic that could kill a large fraction of the human population.

In view of the above, the author has researched and invented a highly effective disinfectant to protect those who are exposed to the pathogenic H5NI virus through direct or direct contact with the said virus.
The author has discovered the chemical composition of a high efficacy disinfectant against H5N1 Virus by synergistically blending a major portion of alcohol [16] and a minor portion of twin-chain quaternary ammonium compound, [14,15,] Spearmint and Peppermint essential oil [18,19,20] and alcohol ethoxylate surfactant [17] with water.
In an embodiment of the invention the ethyl alcohol (C_2H_5OH) [16] or methyl alcohol is used as a co-disinfectant.
In another embodiment of the invention Didecyl Dimethyl Ammonium Chloride [14,15] is used as the main disinfecting agent against H5N1.
In yet another embodiment of the invention alcohol ethoxylate surfactant [17]is used a cleaning agent.
In yet another embodiment of the invention the peppermint essential oil [18,19,20,] is used a synergistic co-disinfectant.
In yet another embodiment of the invention water [21]is used as co-carrier and to increase the solubility properties of the synergistically blended chemical raw materials. It is also used to create an exothermic reaction with alcohol during manufacturing process to enhance the solubility of the essential oil in the said chemical composition.
The foregoing embodiments are susceptible to considerable variation in its practice, limited to the specific exemplifications set forth hereinabove. Rather, the foregoing embodiments are not within the spirit and scope of the appended claims, including the equivalents thereof available as matter of law.
The author Nelson Cheng's intent is to dedicate all disclosed embodiments to the public to save lives should a pandemic strikes the globe.

Detailed Description of The Disinfectant Efficacy of said Invention.

1) A chemical composition of a fast-acting disinfectant and cleaner against highly pathogenic H5N1 Virus comprising of all the steps of a to f sequentially:
a. First mixing alcohol and solvent in the mixing tank to create an exothermic reaction [21] for the purpose of enhancing the solubility of oil based chemical product in said mixture.
b. Second adding quaternary ammonium compound [14,15] in said mixture to increase the anti-viral and antimicrobial properties of said mixture at a temperature range of 32ºC to 35ºC.
c. Third adding spearmint and peppermint [18,19,20] essential oil to said mixture to further enhance the synergistic effect of the anti-viral and antimicrobial properties of said mixture at a temperature range of 32ºC to 35ºC.
d. Fourth adding alcohol ethoxylate surfactant [17] to increase the cleaning properties of said mixture at a temperature range of 32ºC to 35ºC.
2) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus mentioned in 1a, wherein the alcohol used is Ethyl Alcohol or Methyl Alcohol [16] is added to the mixing tank containing solvent at temperature range of 20ºC to 25ºC,and stir said mixture for 5 to 10 minutes to allow

exothermic reaction to take place till the temperature of the said mixture reaches 32ºC to 35ºC before adding the quaternary ammonium compound, essential oil and non-ionic surfactant is Alcohol Ethoxylate is added to the said chemical composition.

3) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as in mentioned point 2, wherein the alcohol used is Ethyl Alcohol C_2H_5OH [16] to enhance the synergistic effect of the anti-viral and antimicrobial properties.

4) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in 1b, wherein the quaternary ammonium compound is twin-chain quaternary ammonium compound [14,15]is added to the above said chemical composition at 32ºC to 35ºC to increase its anti-viral properties of the chemical composition.

5) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in point 4, wherein the twin-chain quaternary ammonium compound used is Didecyl Dimethyl Ammonium Chloride [14,15] due to its high viral efficacy against H5N1 virus.

6) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in 1c, wherein the Essential Oil is Peppermint or spearmint [18,19,20] is added to the said chemical composition at 32ºC to 35ºC and allowed to mix for 10 to 15 minutes to further enhance the synergistic effect of the anti-viral and antimicrobial properties of said composition.

7) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in 1d, wherein the non-ionic surfactant is Alcohol Ethoxylated [17] at a temperature range of 32ºC to 35ºCto increase the cleaning and emulsifying properties of said chemical composition.

8) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in 1a, wherein the solvent used is water [21] due to its excellent solvency for said chemical composition and to create an exothermic reaction with alcohol to enhance the solubility of the essential oil.

9) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in point 8, wherein the water content ranges from 10% to 80% wt/wt.

10) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in point 3 wherein the alcohol ethyl alcohol is used due to its excellent compatibility with said chemical composition.

11) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in point 10, wherein the composition content of ethyl alcohol ranges from 10% to 80% wt/wt.

12) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned point 5 wherein the composition content of Didecyl Dimethyl Ammonium Compound ranges from 0.5% to 5% wt/wt.

13) A chemical composition of a fast acting-disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in point 6 wherein the essential oil used is peppermint to enhance the anti-viral and antimicrobial properties of the said composition.

14) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in point 13 wherein the peppermint used is Mentha Pepertia due to its excellent

15) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in point 13 wherein the content composition of the peppermint ranges from 0.1% to 5% wt/wt.

16) A chemical composition of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in point 1 wherein the composition prepared comprising:

a) 10-80 wt % Ethyl Alcohol
b) 0.5- 5 wt % of Quaternary Ammonium Compound
c) 0.1- 3 wt % of Essential Oil
d) 0.5- 3 wt % Alcohol Ethoxylate
e) 10- 50 wt % Solvent

17) A chemical of a fast-acting disinfectant and cleaner against pathogenic H5N1 Virus as mentioned in point 16 wherein the composition prepared comprising:

a) 70% of Ethyl Alcohol
b) 1.5% of Quaternary Ammonium Compound
c) 4% of Essential Oil
d) 2.5%Alcohol Ethoxylate
e) 22% of water

In accordance with this journal article, its' objective is to provide the test procedures to ascertain the

disinfectant efficacy of said invention against Avian Influenza H5N1 Virus.
A sample of Legionella-X disinfectant was sent to Division of Pathology, Department of Clinic, Reproduction Pathology, Pathology Faculty Veterinary Medicine Bogor Agriculture University, Republic of Indonesia for testing its efficacy against H5N1 Virus. [27,28,29,30,31,32]

The method and test procedures are herein described.
The purpose of the test is to ascertain the efficacy of Legionella-X disinfectant against Avian Influenza H5N1 virus.

1.1 Observation Parameter
The test parameter is based on the observation of the percentage of death virus after the introduction of said invention/disinfectant.

2.0 Test Method [27,28,29,30,31,32]
Using Isolated AI H5N1 virus from Tasikmalaya 2005, collected from Microbiology Department of Faculty of Veterinary, Bogor Institute of Agriculture (IPB). The test was done in the unit of the Integrated Services of Medicine of Microbiology, Faculty of Veterinary IPB. Five live egg embryos Specific Pathogen Free (SPF) were used as medium for the test.

The haemagglutination-inhibition (HAI) assay is a traditional method for assessing immune responses to influenza virus haemagglutinin (HA) and for identifying influenza virus field isolates. The HA protein on the surface of influenza virus agglutinates erythrocytes.

2.1 Test Procedure [27,28,29,30,31,32]
Three components were used; AI H5N1 Virus, Live Egg Embryos and said disinfectant 100% concentration. The preparation of the disinfectant solution was done by diluting 1 part of said disinfectant to 1part distilled water by weight. Subsequently, 2 ml of AI H5N1 Virus (10^{9} EID_{50}) was introduced in to the said disinfectant solution and then the mixture was incubated for 15 minutes at 37ºC.
0.2 ml of said mixture was then injected into 11 days old live embryo via allantois and kept in the incubator at 37ºC, observation was then carried out daily till the death of embryo. The liquid of the allantois was taken out for rapid test, HA/HI using AI standard serum.
3.0 Results
Based on observation all the embryos died two days after the introduction of the mixture of said disinfectant and H5NI Virus. The liquid from the allantois of the death embryo was then taken for rapid test HA/HI using AI standard serum.
The results as tabulated below:

Table1. The Efficacy Result of said invention/disinfectant against AI H5NI Virus

Concentration	Dilution	Percentage of Inactive Virus AI H5N1 (%)
100	1:1	100

Based on Table 1, the said invention/disinfectant has the ability to inactivate 100% of AI H5NI virus.

4.0 Conclusion
Based on the said efficacy test, the said invention/disinfectant is highly effective against Avian Influenza H5N1 Virus.

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