The Efficacy on Inactivation of Newcastle Disease Virus by Using Different Types of Commercially Available Detergents

Thet Naing Aye[1] Naruepon Kampa[2] Kingkarn Sarachu[3] Kanlaya Chuachan[3] Tawatchai Pohuang[3,4*]

Abstract

Objective - To evaluate the efficacy of commercially available detergents on inactivation of Newcastle disease virus

Materials and Methods - The four commercially available detergents including powdered detergent, shampoo, liquid soap and dishwashing liquid were randomly selected and tested for the efficacy on inactivation of the Newcastle disease virus (NDV) by using 0.3%, 0.6%, 0.9%, 1.8%, and 3.6% of each detergent at the inactivation time of 5, 10 and 15 min. The RNA of treated NDV was detected by reverse transcriptase-polymerase chain reaction. The infectivity of treated NDV was detected by inoculated into embryonated chicken eggs. After that, the allantoic fluid was collected and confirmed for the presence of NDV by hemagglutination test and hemagglutination inhibition test.

Results - The infectivity of NDV was inhibited in the treatment including 0.3% of powdered detergent at 10 min, 0.6%, 0.9%, 1.8%, 3.6% of powdered detergent at 5 min, 3.6% of liquid soap at 15 min and 3.6% of dishwashing liquid at 10 min. All treatments which could inhibit the infectivity of NDV were positive by using reverse-transcriptase polymerase chain reaction for detection of NDV, except for the treatment with 3.6% of powdered detergent at 15 min.

Conclusion - This study indicated that powdered detergent, liquid soap and dishwashing liquid were effectively on inactivation of NDV.

Keywords: Newcastle disease virus, inactivation, detergent

[1]Master of Science in Interdisciplinary Veterinary Medicine (International Program), [2]Department of surgery and theriogenology, [3]Department of Medicine, [4]Research Group of Preventive Technology in Livestock, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen 40002.

***Corresponding author** E-mail: ptawat@kku.ac.th

ประสิทธิผลในการทำลายเชื้อไวรัสนิวคาสเซิล ด้วยการใช้สารซักฟอกชนิดต่าง ๆ ที่มีจำหน่ายในท้องตลาด

Thet Naing Aye[1] นฤพนธ์ คำพา[2] กิ่งกาญจน์ สาระชู[3] กัลยา เจือจันทร์[3] ธวัชชัย โพธิ์เฮือง[3,4*]

บทคัดย่อ

วัตถุประสงค์ เพื่อทดสอบประสิทธิผลในการทำลายเชื้อไวรัสนิวคาสเซิลของสารซักฟอกชนิดต่างๆ ที่มีจำหน่ายในท้องตลาด

วัสดุ อุปกรณ์ และวิธีการ ทำการสุ่มเลือกสารซักฟอกที่มีจำหน่ายในท้องตลาด 4 ชนิดได้แก่ ผงซักฟอก ยาสระผม สบู่เหลว และน้ำยาล้างจาน จากนั้นทดสอบความสามารถในการทำลายเชื้อไวรัสนิวคาสเซิลโดยใช้ความเข้มข้น 0.3%, 0.6%, 0.9%, 1.8% และ 3.6% ใช้เวลาในการทำลายเชื้อ 5, 10 และ 15 นาที ตรวจสอบผลการทำลาย RNA ของเชื้อด้วยปฏิกิริยาลูกโซ่โพลีเมอร์เรสแบบย้อนกลับ และตรวจสอบผลการทำลายเชื้อด้วยการฉีดเชื้อที่ผ่านสัมผัสกับสารซักฟอกดังกล่าวเข้าไข่ไก่ฟัก แล้วเก็บ allantoic fluid มาตรวจด้วยวิธี hemagglutination test และ hemagglutination inhibition test

ผลการศึกษา พบว่าเชื้อไวรัสนิวคาสเซิลถูกทำลายด้วยการใช้ผงซักฟอกความเข้มข้น 0.3% ใช้เวลาในการทำลายเชื้อตั้งแต่ 10 นาที การใช้ผงซักฟอกความเข้มข้น 0.6%-3.6% ใช้เวลาในการทำลายเชื้อตั้งแต่ 5 นาที การใช้น้ำยาล้างจานความเข้มข้น 3.6% ใช้เวลาในการทำลายเชื้อตั้งแต่ 10 นาที และการใช้สบู่เหลวความเข้มข้น 3.6% ใช้เวลาในการทำลายเชื้อตั้งแต่ 15 นาที นอกจากนี้ยังพบว่าเชื้อไวรัสนิวคาสเซิลที่ถูกทำลายด้วยสารซักฟอกดังกล่าวยกเว้นการใช้ผงซักฟอกความเข้มข้น 3.6% ใช้เวลาในการทำลายเชื้อ 15 นาที ยังสามารถตรวจพบสารพันธุกรรมของเชื้อได้ด้วยปฏิกิริยาลูกโซ่โพลีเมอร์เรสแบบย้อนกลับ

ข้อสรุป การศึกษาในครั้งนี้แสดงให้เห็นว่าผงซักฟอก น้ำยาล้างจาน และสบู่เหลวสามารถทำลายเชื้อไวรัสนิวคาสเซิลได้

คำสำคัญ: เชื้อไวรัสนิวคาสเซิล การทำลาย สารซักฟอก

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[1]Master of Science in Interdisciplinary Veterinary Medicine (International Program) [2]ภาควิชาศัลยศาสตร์และวิทยาการสืบพันธุ์ [3]ภาควิชาอายุรศาสตร์ [4]กลุ่มวิจัยเทคโนโลยีการป้องกันโรคในปศุสัตว์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยขอนแก่น จังหวัดขอนแก่น 40002

[*]**ผู้เขียนที่ให้การติดต่อ** อี-เมล์: ptawat@kku.ac.th

Introduction

Newcastle Disease (ND), one of the most infectious viral diseases of poultry, is present worldwide and affects many species of birds causing economic losses in the poultry sector in both of developed and developing countries. Moreover, its outbreak severely impacts commercial productivity and the international trade in poultry and poultry products. ND was first discovered in Java, Indonesia, and Newcastle-upon-Tyne, England, during the mid-1920s [1]. It is caused by avian paramyxovirus serotype-1 (APMV-1), a member of the genus *Avulavirus* within the *Paramyxoviridae* family [2]. NDV is enveloped and contains a negative-sense, single stranded ribonucleic acid (RNA) genome of 15,186 nt length [3]. Based on the severity of the disease in chickens, NDV strains are grouped into three main pathotypes: lentogenic, mesogenic and velogenic [4]. Among many species of birds, chickens are the most susceptible host, in which the severity of the disease may vary from mild infection to a severe form causing 100% mortality. NDV is highly transmissible, the birds are normally infected through direct contact with diseased or carrier birds, but the virus can also be carried on contaminated objects such as chicken or egg crates, feed, water, vehicles, dust and clothing. As such, it can also spread through several routes by the movement of live poultry and their products and the movement of people and equipment from poultry or aviary facilities [5].

NDV belongs to category A, in which all enveloped viruses of intermediate to large size, is generally not considered to be resistant to chemical inactivation due to the relatively easy disruption of the lipid envelope by most chemical biocides [6,7]. In spite of mass vaccination programs and improved hygienic measures, ND is still a common disease of poultry. During an outbreak, disinfection is not carried out properly, infection will persist and concurrent damage to the poultry production. Contaminated clothing, equipment and worker could easily act as a source of infection for uninfected chicks [5]. For this reasons, the use of virucidal agents has been an integral component of ND control programs, notwithstanding, the appropriate virucidal agents must be selected based on the susceptibility of the target virus and harmless to the human. Detergents such as powdered detergent, shampoo, liquid soap and dishwashing liquid are commonly used in daily life. Although, the mainly purpose on using those of detergent is focused on cleansing procedures, some of them are suggested by the manufacturer as anti-bacterial detergent. They are considered to be efficacious against the pathogens due to their surfactant property [8]. In this study, different types of main surfactant are found in the tested

detergents including anionic surfactant in powdered detergent, sodium lauryl sulfate in shampoo, sodium laureth sulfate in liquid soap and linear alkylbenzene sulfonate in dishwashing liquid. There were the reports on inactivation of herpes simplex virus [9] and human immunodeficiency virus [10] by using Sodium lauryl sulfate. However, there is a lack of information on inactivation of NDV by using commercially available detergents. This study, therefore, was designed to evaluate the efficacy of commercially available detergents on inactivation of NDV. The result of this study would be useful in ND control in poultry production; especially, for the family or village flocks that commercial disinfectants are difficult to provide and the price is too expensive.

Materials and Methods

Virus propagation and titration: NDV was propagated in 10-day-old embryonated chicken eggs (ECEs). Infectious allantoic fluid (AF) was harvested and made ten-fold virus dilutions. Five, 10-day-old ECEs were inoculated in the allantoic cavity with 100 µl per egg of each ten-fold dilution of the virus. Inoculated ECEs were incubated at 37°C and were candled twice a day. Five days after inoculation, AF was harvested and examined for NDV infection by using hemagglutination (HA) test [11]. The virus titers determined as egg infective dose 50% (EID_{50})/0.1 ml were evaluated according to the Reed and Muench method [12]. The AF containing viruses were kept at -70°C till further use.

Detergents: The four commercially available detergents were randomly selected and tested for the efficacy on inactivation of the NDV including powdered detergent (PAO®, Lion Corporation, Thailand), shampoo (Pantene®, The Procter and Gamble manufacturing, Thailand), liquid soap (Dettol®, Reckitt Benckiser, China), dishwashing liquid (Sunlight®, Unilever, Thailand). In order to determine their embryo toxic effect, the preliminary study was performed by using inoculated the 10-day-old ECEs in five replications with the required concentration (0.3%, 0.6%, 0.9%, 1.8%, and 3.6%) of each detergent. Embryo toxic effect was not observed in all of four detergents at 0.9%.

Virus Inactivation: NDV stock AF was diluted with PBS to get the initial concentration of $10^{5.6}$ EID_{50}/ml. The treatments were composted of 1 ml of each diluted detergent (0.6%, 1.2%, 1.8%, 3.6%, and 7.2%) were mixed with 1 ml of $10^{5.6}$ EID_{50}/ml of NDV solution in order to get the final concentrations (0.3%, 0.6%, 0.9%, 1.8% and 3.6%) and then placed at room temperature for 5,

10, 15 min. In addition, 1 ml of NDV mixed with 1ml sterile distilled water (virus control 1 and 2), 1 ml of NDV mixed with 1 ml of 2% glutaraldehyde and PBS mixed with 1 ml sterile distilled water were served as the control.

Virus Isolation and Detection: After 5, 10, 15 min incubation period, the virus mixed with the detergent at concentration of 1.8% and 3.6%, the virus mixed with glutaraldehyde 2%, and the virus control 2 were diluted ten-fold before inoculated into the ECEs to avoid their embryo toxic effect. The remaining virus mixed with detergents could directly inoculate in to the ECEs. Each treatment (0.2 ml) was inoculated into 10-day-old ECEs in five replications and candled twice a day for 5 days. Following incubation at 37°C, embryos that died within 24 hr were discarded. At 5 days after inoculation, AF was collected from all of the inoculated eggs and the HA test was performed as described by OIE [11]. AF which was positive by HA test will be further tested for the hemagglutination inhibition (HI) test using specific NDV antiserum. The ability on inactivation of the virus by treatments was indicated by the survival of embryo, negative result of HA activity of the AF.

Reverse-transcriptase polymerase chain reaction: In order to detect nucleic acid destroying activity of the detergents, before inoculation into ECEs, the virus mixed with detergent were subjected to RNA extraction by using Viral Nucleic Acid extraction kit (Real Biotech, Taiwan). The RNA from the treatment which had negative HA result was subjected to reverse-transcriptase polymerase chain reaction (RT-PCR). The reaction was performed by using Access Quick™ RT-PCR System (Promega, USA) with forward primers FOP1: 5´-TACACCTCATCCCAGACAGGGTC-3´ and reverse primers RENDV371: 5´GAGAGCTACACCACCGATAATGG-3´ [13]. Cycling conditions included a reverse transcription step with 48°C for 45 min followed by 94°C for 5 min. The amplification steps included an initial denaturation (94°C for 30 sec), annealing (60°C for 30 sec), polymerization (72°C for 30 sec) for 35 cycles, and a final polymerization (72°C for 10 min). Then, RT-PCR products were subjected to agarose gel electrophoresis using a 2% agarose gel followed by staining with ethidium bromide (0.5 µg/ml) and visualized by ultraviolet trans-illuminator.

Results

Virus Inactivation: The result was shown in Table 1. In the absence of detergent treatments, NDV was still alive in sterile distilled water until 15 min. NDV was inactivated by the treatment

with 0.3% of powdered detergent at 10 min. With the higher concentration of powdered detergent (0.6%, 0.9%, 1.8%, 3.6%) NDV was inactivated at least 5 min of contact times in this study. On the other hand, using the highest concentration (3.6%) of shampoo for 15 min was not effective on the inactivation of NDV. The effects of liquid soap and dishwashing liquid in the same concentration (3.6%) on the inactivation of NDV were observed at the contact times of 15 and 10 min, respectively. Glutaraldehyde (1%) could effectively on inactivation of NDV at 5 min contact time.

RT-PCR: The further test was performed to know the deleterious effect of the detergents on viral nucleic acid by using RT-PCR technique. The results showed that the RNA of NDV which was inactivated by 3.6% of liquid soap after 15 min and dishwashing liquid after 10 min exposure time could be detected by RT-PCR (Figure1). Although the infectivity of NDV was absent after treatment with 0.3%, 0.6%, 0.9%, 1.8% of powdered detergent, all treated NDV was positive by RT-PCR (Figure2). In this study the negative results of RT-PCR was only found in the treated samples from 3.6% of powdered detergent at the contact time of 15 min (Figure2).

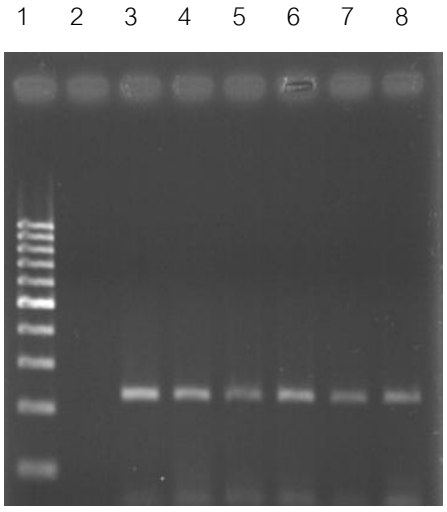

Figure 1. RT-PCR Products After Dishwashing Liquid and Liquid Soap Treatment.

Lane assignments: lane 1, 100 bp marker; lane2, negative control; lane 3 and 4, NDV control 1and 2; lane 5 and 6, NDV after 10 and 15 min 3.6% dishwashing liquid treatment; lane 7 and 8, NDV after 10 and 15 min 3.6% liquid soap treatment.

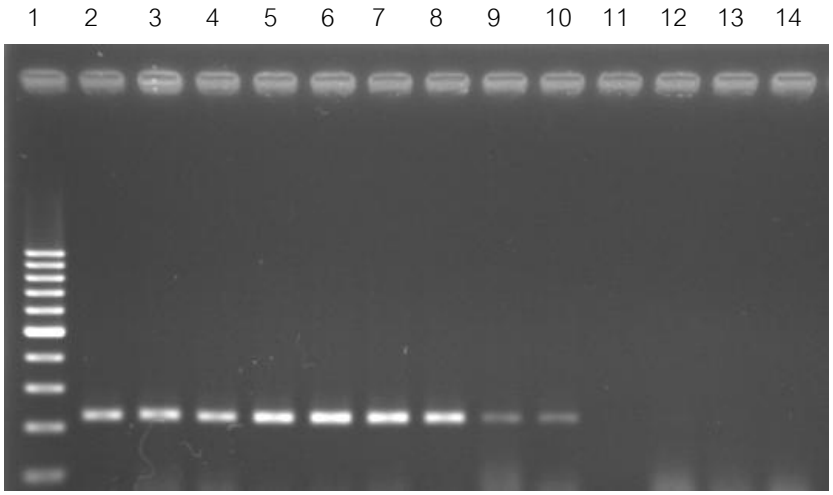

Figure 2. RT-PCR Products After Powdered detergent and Glutaraldehyde Treatment.

Lane assignments: lane 1, 100 bp marker; lane2 and 3, NDV after 10 and 15 min 0.3% powdered detergent; lane 4 and 5, NDV after 5 and 15 min 0.6% powdered detergent; lane 6 and 7, NDV after 5 and 15 min 0.9% powdered detergent; lane 8 and 9 NDV after 5 and 15 min 1.8% powdered detergent; lane 10 and 11 NDV after 10 and 15 min 3.6% powdered detergent; lane 12, 13 and 14 NDV after 5, 10 and 15 min 1% glutaraldehyde.

Discussion

In our experiment, 0.3%, 0.6%, 0.9%, 1.8%, 3.6% of powdered detergent had the effect on inhibition of NDV infectivity. The effect of powdered detergent on inactivation of virus had been reported such as the inactivation of low pathogenic avian influenza virus (LPAIV) by using 6 g/L of powdered detergent with peroxygen [14]. However, another researcher reported that the powdered detergent was shown to be ineffective at completely disinfecting LPAIV [15]. All tested concentrations showed positive HA results. So, they assumed that the efficacy of the detergent appeared to be concentration dependent and the concentration higher than 6 g/L may be effective at inactivation of LPAIV. We also observed that the completely disinfecting NDV by using 0.3% of powdered detergent occurred at the contact time of 10 min. Base on this result, the efficacy of the detergent appeared to be contact time dependant. We decided to use the contact time for NDV inactivation as 5, 10 and 15 min because the report of Muhammad et al. [16] showed that complete inactivation of avian influenza virus (AIV) occurred after using 0.1% of detergent (Surf Excel®) for 5 min contact time. The good efficacy of the powdered detergent may be due to a destructive effect of the surfactants on the virus envelope which enables the virus particle to recognize the host cell and to adhere to it. AIV and NDV are the

enveloped viruses; therefore mechanism on inactivation of the virus by the detergents may be the same.

Virus inactivation activity of commercially available soap (Lifebuoy®) was studied by Muhammad et al. [16]. They observed that 0.1%, 0.2% and 0.3% of soap (Lifebuoy®) can destroy infectivity of AIV after 5 min contact time while 0.05% was not enough to kill virus after 45 min. The efficacy in denaturing protein was occurred by the presence of hydroxide ion in alkalis, is also related to environmental temperature and concentration [17]. In this experiment, even though we used liquid soap, similar results were obtained. NDV showed resistance to inactivation with 0.3%, 0.6%, 0.9%, 1.8% of liquid soap after 15 min and 3.6 % after 10 min contact time, whereas 3.6% concentration was effective at 15 min.

Specific studies on the efficacy of shampoo and dishwashing liquid are not available in the literature. This is perhaps the first report on the efficacy of shampoo and dishwashing liquid against NDV as disinfectant. Shampoos are cleaning compounds, generally comprise a mixture of ingredients as detergents and soaps and designed to remove oils and debris from hair. Shampoo evaluated in this study was not sufficient to inactivate NDV even at the highest concentration 3.6% at contact time of 15 min. Strain of the virus, exposure time, quantity of the virus, nature of the medium and interaction between the treatments are also contributing factors in viral destruction [5]. It is therefore inferred that different brand of shampoo may be effective at inactivation of NDV at high concentration, however, further testing is required to confirm. When evaluating the virucidal performance of the dishwashing liquid, these findings indicate that 3.6% was able to inactivate NDV fully within 10 min while 5 min contact time was not effective. However, at low concentrations such as 0.3%, 0.6%, 0.9%, 1.8% was not sufficient in killing NDV at 15 min contact time at the same environmental temperature. Hence, dishwashing liquid is considered to be concentration and exposure time dependent disinfectant.

The powdered detergent, shampoo, liquid soap and dishwashing liquid are generally used for cleaning procedures. They are considered to be efficacious against the virus due to their surfactant property that can effect on the lipid components of virus particles [8]. Soaps and detergents generally comprise a mixture of ingredients including surfactants, lather enhancers [18]. Main surfactant that composed in powdered detergent, shampoo, liquid soap and dishwashing liquid used in this work was difference; including anionic surfactant in powdered detergent, sodium lauryl sulfate in shampoo, sodium laureth sulfate in liquid soap and linear

alkylbenzene sulfonate in dishwashing liquid. The mechanism by which surfactant inactivates viral systems probably involves on the denaturation of proteins or lipid envelope with subsequent degradation of viral capsid proteins [19]. Known surfactant such as quaternary ammonium compounds (QAC), are compounds comprised of a hydrophilic and a lipophilic portion. Their mode of action against bacteria is largely described as cell membrane disruption and loss of potassium ions. The viruses specifically lipophilic-enveloped are susceptible to QACs. The mechanism of action of QACs involves protein denaturation and dissociation of enzymes [20] and interaction with lipids [21].

Elhafi et al [22] reported that import of poultry viruses for identification by molecular technique into a country with strict importation controls has been addressed, especially in the United State. Similarly, import of the samples from countries outside the United Kingdom is restricted. The problem occurred due to the risk of accidentally introducing the infectious pathogens into those countries. The results obtained from this experiment showed that powdered detergent, liquid soap and dishwashing liquid were effective on inactivation of NDV when the appropriate concentration and contact time were selected. Moreover, in the treatments that allowed the stability of nucleic acid of NDV could be detected by RT-PCR. Herein, we suggested that these appear to be the satisfactory detergents for inactivating NDV while preserving its nucleic acid for molecular identification. However, viral RNA of NDV was not detected by using RT-PCR in the treatment with 3.6% of powdered detergent for 15 min while for 5 and 10 min were detected. Similar results were obtained by Dee et al [23]. The viral RNA of porcine reproductive and respiratory syndrome virus was detected in 2 out of 19 swabs at 60 min and 0 out of 19 swabs at 90 min following treatment with glutaraldehyde-quaternary ammonium chloride. They assumed that a negative PCR result could be due to multiple factors, including the diagnostic sensitivity of the test, degradation of viral RNA in the sample through prolonged contact with the disinfectant, interference of the disinfectant with the PCR assay, or the results of a truly efficacious disinfectant that not only rendered the virus inactive, but also degraded its nucleic acid.

We concluded from our findings that powdered detergent at 0.3% for 10 min or 0.6% or higher for 5 min contact time could inactivate NDV. Liquid soap and dishwashing liquid could inactivate NDV by using at the concentration 3.6 % for 15 and 10 min contact time, respectively.

However, at the highest concentration and the highest contact time, shampoo could not inactivate NDV in this study.

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References

1. Doyle TM. A hitherto unrecorded disease of fowls due to a filter-passing virus. *J Comp Pathol*. 1927; 40: 144-169.
2. Mayo MA. A summary of taxonomic changes recently approved by ICTV. *Arch Virol*. 2002; 147: 1655-1663.
3. Krishnamurthy S, Samal SK. Nucleotide sequences of the trailer, nucleocapsid protein gene and intergenic regions of Newcastle disease virus strain Beaudette C and completion of the entire genome sequence. *J Gen Virol*. 1998; 79: 2419-2424.
4. Alexander DJ, Manvell RJ, Frost KM, Pollitt WJ, Welchman D, Perry K. Newcastle disease outbreak in pheasants in Great Britain in May 1996. *Vet Rec*. 1997; 140: 20-22.
5. Alexander DJ. Newcastle disease. In: Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE (editors). *Diseases of Poultry*. 11th ed. Iowa State Press; 2003. p. 64-87.
6. Klein M and Deforest A. Principles of viral inactivation. In: Block SS, (editor). *Disinfection, Sterilization and Preservation*. 3rd ed. Lea and Febiger: Philadelphia; 1983. p. 422-434.
7. Prince HN and Prince DL. Principles of viral control and transmission In: Block SS, (editor). *Disinfection, Sterilization and Preservation*. 5th ed. Lippincott: Williams and Wilkins; 2001. p. 543-573.
8. Ausvetplan [Internet]. Australian Veterinary Emergency Manual Plan Avian Influenza Updated Interim Draft (1,891), 3rd ed., c2005; Version 3.1 [cited 2013 Sep 12]. Available from: http://www.animalhealthaustralia.com.au/aahc
9. Piret J, Roy S, Gagnon M, Landry S, Désormeaux A, Omar RF, Bergeron MG. Comparative study of mechanisms of herpes simplex virus inactivation by sodium lauryl sulfate and n-lauroylsarcosine. 2002; 46:2933-42.

10. Bestman-Smith J(1), Piret J, Désormeaux A, Tremblay MJ, Omar RF, Bergeron MG. Sodium lauryl sulfate abrogates human immunodeficiency virus infectivity by affecting viral attachment. 2001; 45:2229-2237.
11. Office International des Epizooties (OIE). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2008 [Internet] [revised 2008 July 17; cited 2009 January 20]. Available from: http://www.oie.int/eng/normes/mmanual/2008/pdf/2.03.14_ NEWCASTLE_DIS.pdf.
12. Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. *Am J Hyg*. 1938; 27: 493-497.
13. Pohuang T, Chuachan K, Sarachu K, Sukolapong V. Effect of microwave, ultraviolet light, Clorox® and Dettol® on inactivation of Newcastle Disease virus but allowing detection of its RNA. *KKU Vet J*. 2010; 20: 165-177.
14. Lombardi ME, Ladman BS, Alphin RL, Benson ER. Inactivation of avian influenza virus using common detergents and chemicals. *Avian Dis*. 2008; 52: 118-123.
15. Alphin RL, Johnson KJ, Ladman BS, Benson ER. Inactivation of avian influenza virus using four common chemicals and one detergent. *Poult Sci*. 2009; 88 :1181-1185.
16. Muhammad AS, Muhammad A, Sajid H, Shamsul H. Avian influenza virus (H5N1); effects of physico-chemical factors on its survival. *Virol J*. 2009; 6: 38.
17. Jeffery DJ. Chemicals used as disinfectants: active ingredients and enhancing additives. *Rev Sci Tech*. 1995; 14: 57-74.
18. Wolf R, Wolf D, Tuzun B, Tuzun Y. 2001. Soaps, shampoos, and detergents. *Clin Dermatol*. 2001; 19: 393-397.
19. Mallaird JY. Viricidal activity of biocides. In: Fraise AP, Lambert PA, Mallaird JY, (editors). *Principles and Practice of Disinfection Preservation & Sterilization*. Blackwell Publishing Ltd, Oxford: UK; 2004. p. 272-323.
20. Merianos JJ. Surface-Active Agents. In: Block SS, (editor). *Disinfection, Sterilization and Preservation*. 5th ed. Lippincott: Williams and Wilkins; 2001. p. 283-320.
21. Lambert PA. Mechanismsof action of biocides. In: Fraise AP, Lambert PA, Mallaird JY, (editors). *Principles and Practice of Disinfection Preservation & Sterilization*. Blackwell Publishing Ltd, Oxford: UK; 2004. p. 139-153.

22. Elhafi G, Naylor CJ, Savage CE, Jones RC. Microwave or autoclave treatments destroy the infectivity of infectious bronchitis virus and avian pneumovirus but allow detection by reverse transcriptase-polymerase chain reaction. *Avian Pathol*. 2004; 33: 303-306.
23. Dee SA, Deen J, Burns D, Douthit G, Pijoan C. An assessment of sanitation protocols for commercial transport vehicles contaminated with porcine reproductive and respiratory syndrome virus. *Canadian J Vet Res*. 2004; 68: 208–214.

Table 1. The Efficacy of Detergent Treatment on Inactivation of NDV.

Detergent	Concentration (%)	Number of HA and HI positive/ inoculated egg			Result on inactivation of NDV		
		Inactivation time (min)			Inactivation time (min)		
		5	10	15	5	10	15
Powdered detergent	0.3	2/5	0/5	0/5	+[C]	-[D]	-
	0.6	0/5	0/5	0/5	-	-	-
	0.9	0/5	0/5	0/5	-	-	-
	1.8	0/5	0/5	0/5	-	-	-
	3.6	0/5	0/5	0/5	-	-	-
Shampoo	0.3	5/5	5/5	5/5	+	+	+
	0.6	5/5	5/5	5/5	+	+	+
	0.9	4/5	3/5	5/5	+	+	+
	1.8	5/5	5/5	5/5	+	+	+
	3.6	5/5	5/5	5/5	+	+	+
Liquid soap	0.3	4/5	5/5	5/5	+	+	+
	0.6	5/5	5/5	5/5	+	+	+
	0.9	4/5	5/5	5/5	+	+	+
	1.8	5/5	5/5	4/5	+	+	+
	3.6	3/5	2/5	0/5	+	+	-
Dishwashing liquid	0.3	5/5	5/5	5/5	+	+	+
	0.6	5/5	4/5	5/5	+	+	+
	0.9	5/5	3/5	4/5	+	+	+
	1.8	4/5	3/5	2/5	+	+	+
	3.6	5/5	0/5	0/5	+	-	-
NDV control1[A]	-	5/5	5/5	5/5	+	+	+
NDV control2[B]	-	5/5	5/5	5/5	+	+	+
Glutaraldehyde	1	0/5	0/5	0/5	-	-	-

[A] this treatment was directly inoculated into embryonated chicken eggs

[B] this treatment was diluted ten-fold before inoculation to embryonated chicken eggs

[C] + = detectable

[D] - = undetectable