

The Neutralizing Effects of ASAP (Colloidal Silver Solution) On the Newcastle Disease Virus.

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ABSTRACT

This work was aimed at testing the effects ASAP Solution, an FAD approved, and carefully engineered, silver nano-particle mineral supplement on New-Castle Disease virus in some infected poultry birds at the National Veterinary Research Institute, Vom (NVRI) Plateau State of Nigeria. The research was designed by randomly blocking (during drug dilution process) thirty five eggs (35) of 9 (nine) day old hen's embryonated eggs into groups of seven (7) based on drug dilutions viz; six (6) drug dilution groups and the control group. Dilutions from 10^{-5} to 10^{-10} were used for the assay. 0.1ml of the respective dilutions was transferred to sterile Bijou bottles labelled with the corresponding drug dilution. Thereafter, equal volumes of 4HA unit concentration of neat suspension of a lentogenic Newcastle disease (ND) virus strain was added to each bottle. Five eggs were labelled for each drug dilution. 0.1ml drug/virus mixtures were randomly inoculated into five (5) eggs bearing the respective drug dilutions. Five eggs were inoculated with 0.1ml each of the virus suspension to serve as virus control. Eggs were incubated at 37°C for 72 hours. Each egg was cut open after incubation and allantoic fluid tested for Heamagglutination reaction using 10% chicken RBC. The results revealed that ASAP colloidal silver solution has neutralizing effects on Newcastle Disease Virus by the Heamagglutination inhibition technique (HAI). Therefore Neutralization titre = $10^{-5.6 \text{ ND}_{50}/0.1\text{mL}}$. This study findings support the conclusions made that the ASAP Colloidal Silver Solution is naturally microcidal (killing harmful bacteria, yeast, fungus, and viruses).

INTRODUCTION

The ASAP Solution® is an engineered silver nano-particle mineral supplement. It includes 10 parts per million (ppm) silver utilizing American Biotech Labs unique patented silver technology. ¹ It is an immune system support, meaning that it is designed to help boost a healthy immune system, to act as a first line of defence. It has been proven safe for daily usage when used as suggested. Hundreds of tests have been completed on the ASAP Solution® at independent government certified laboratories, both domestic and international, as well as numerous universities and government sponsored laboratories ¹. American Biotech Labs is a world leader in silver technology and has a long history with the uses of silver and natural silver supplements. Silver has been used for centuries and has been one of the most effective natural methods in killing bacteria. It can be traced back as far as the Egyptians era. For the past three decades, American Biotech Labs set out to test the ASAP Silver Solution, the most technically advanced silver based product in the world by utilizing a team of Scientists, Doctors, Certified Independent Laboratories, Major Universities, and International Health Consortiums ¹. Through years of bacteria and pathogenic studies, American Biotech Labs' researchers extensively analyzed some of the deadliest

bacteria and viruses known to man. Their research was crucial in the development of the ASAP Silver Solution(tm). They realized that basic elements of bacteria had to be unraveled in order to develop a silver based supplement that could kill the deadliest pathogens known to mankind ². These Silver Sol nanoparticles work as a natural, safe antiseptic for the body. This patented product combines the natural vitality of mother earth's silver with purified water in a specially discovered concentration that blasts pathogens with natural healing frequencies. At 10 ppm, it has been scientifically shown to destroy harmful bacteria, viruses - health robbing pathogens! Regular use helps the immune system fight off low grade, hard-to-detect infections ³. Colloidal silver is a suspension of sub-microscopic metallic silver particles in a colloidal base.

Silver metal is naturally antimicrobial (killing harmful bacteria, yeast, fungus, virus), and yet has no negative effect on human tissue when used in reasonable amounts. It has a natural anti-inflammatory action which reduces pain and promotes natural healing. Silver is extensively used in medicine today. Currently, there are over 80 FDA approved silver based products and more are being approved monthly. Silver is the number one treatment for burns in U.S. burn centers, being administered in the form of gels and creams. New silver coated catheters are used because they stop the urinary tract infections that were commonplace with the old ones. New testing is producing silver heart valves that bacteria cannot damage. To protect us from food poisoning, silver particles are now being put in cutting boards, table tops, surface disinfectants, and refrigerators. Silver is now widely used in clothing, not only for the military, but for the sportsman and businessman. It is woven and impregnated into the fabric to kill bacteria that cause body odour, and even to fix that smell that comes from basketball shoes. It is being used on and in foods, as a decoration and disinfectant. Because of its high conductivity, silver or silver alloys are used in almost every electrical connection in your house, from your toaster to your shaver, computer, hot water heater, car, coffee maker, and even your cell phone. New applications are being engineered daily and include numerous areas in the dental and medical field. A new peer review paper showed that it is likely that a new silver liquid may save your life in the event of an H5-N1 bird flu pandemic. It is safe to say that silver is used almost every day by every man, woman, or child in the civilized world ^{4,5}. The ASAP Colloidal Silver Solution is the only patented colloidal silver in the market. The reason for this is that it has been proven to be such an effective colloidal silver solution that it reaches far beyond any previous colloidal silver products. ASAP Colloidal Silver Solution was approved for patent by the United States Patent Agency. In recent tests conducted by Brigham Young University Microbiology Laboratory, the ASAP Colloidal Silver Solution has been shown to have a greater effect on the immune system than other colloidal silver products ³.

NEWCASTLE DISEASE VIRUS ; Newcastle disease was first discovered in 1926 at Jakarta in Indonesia ⁶. It is a poultry disease that affects mostly avian species industry. Farmers suffer losses due to Newcastle disease virus because of low egg production, loss of weight and deaths ⁶. The virus belongs to the family PARAMYXOVIRIDAE. The virus causes acute, rapid spreading, contagious Nervous and respiratory viral disease of birds of all ages ⁷. The symptoms includes gasping, twisting of the neck, dullness, rough feather and greenish yellow diarrhea----causing lesions in the brain, gastro-intestinal tract and respiratory tract. The morbidity rate is near 100 percent while the mortality rates can be as high as 90% in susceptible birds ⁸.

Newcastle disease virus strains are divided into 4 groups based on the disease they cause in their host birds; the velogenic strains which are most virulent followed by the mesogenic strains, then the mild form also known as the lentogenic strains. The fourth group is known as the a virulent strains ⁹. The epizootic spread to the northern English coast around Newcastle gave us idea on where the name was derived ^{10,11}. In some countries such as New Guinea, Australia, only the virulent strains of the V4 type are recognized ¹². The disease is known to have spread over to most parts of the world except the Arctic and Antarctic regions. Recently in 1992, there was an outbreak in Australia to break the old outbreak in 1936. In Nigeria, the disease was first discovered in Eastern Nigeria ¹³ and was confirmed in Vom by post Mortem examination. In 1952, an outbreak was confirmed from Ibadan, Kano and Lagos ^{14,15,16}, and since then, the disease has spread to different parts of the country ^{14, 17, 18, 6, 19, 9}. The disease has no treatment and the major source of control in Nigeria today is by vaccination using the three types of vaccines produced at the National Veterinary Research Institute, Vom ²⁰.

HYPOTHESES

Null Hypothesis (Ho):

ASAP has no neutralizing effect on the Newcastle Disease Virus and as such the neutralizing dose is not significant.

Alternate Hypothesis (Ha):

ASAP has a neutralizing effect on the Newcastle Disease Virus and as such the neutralizing dose is significant.

Thirty five eggs (35) of 9 (nine) day old hen's embryonated eggs were obtained from the poultry unit of the National Veterinary Research Institute, Vom, Jos. Plateau State, Nigeria. They eggs were candled to ensure they are alive and fit for the study; GROUPING OF EGGS

The eggs were grouped into seven (7) based on drug dilutions viz;

Group one (1): The control group

Group two (2): The group with drug dilution of 10^{-5}

Group three (3): The group with drug dilution of 10^{-6}

Group four (4): The group with drug dilution of 10^{-7}

Group five (5): The group with drug dilution of 10^{-8}

Group six (6): The group with drug dilution of 10^{-9}

Group seven (7): The group with drug dilution of 10^{-10}

Each group consists of five (5) eggs respectively. Each egg was cut open after incubation and allantoic fluid tested for Heamagglutination reaction using 10% chicken RBC(Red Blood Cell). A total number of thirty five eggs were used for this study. They were divided into seven (7) groups, i.e. ' six (6) drug dilution groups and the control group'. A ten-fold serial dilution of the drug was tested for neutralization i.e. a 10^{-1} to 10^{-10} of the colloidal silver solution using PBS as diluents. Dilutions from 10^{-5} to 10^{-10} were used for the assay. 0.1ml of the respective dilutions was transferred to sterile bijou bottles labelled with the corresponding drug dilution. Thereafter, equal volumes of 4HA unit concentration of neat suspension of a lentogenic Newcastle disease (ND) virus strain was added to each bottle. Bottles were gently agitated to mix their contents and kept on ice ready for inoculation. Egg inoculation THIRTY (30) 9 (Nine) day old Hen's embryonated eggs were prepared for inoculation and five eggs were labelled for each drug dilution. 0.1mL drug/virus mixtures were inoculated into five (5) eggs bearing the respective drug dilutions. Five eggs were inoculated with 0.1mL each of the virus suspension to serve as virus control. Eggs were incubated at 37°C for 72 hours.

SOURCE OF THE DRUG; From Doctor Simon Agwale, the Director of innovative biotechnology in the united states of America.

RESULTS

Each egg was cut open after incubation and allantoic fluid tested for Heamagglutination reaction using 10% chicken RBC. All virus control eggs tested positive for Heamagglutination reaction, indicating the presence of ND virus. Where allantoic fluid in the egg agglutinated RBC, it indicated that the drug failed to neutralize the virus. Where the fluid did not agglutinate RBCs, it showed that the drug has neutralized the virus.

TABLE A

Drug Dilution	HA Negative Number of Eggs neutralized	HA Positive Number of Eggs not neutralized	Accumulated numbers			Percentage neutralized A/A+B X 100 (%)
			Neutralized (A)	Not Neutralized (B)	Total Number tested (A+B)	
10 ⁻⁵	3	2	5	2	7	5/7 x100 = 71.4
10 ⁻⁶	2	3	3	5	8	3/8 x100 =37.5
10 ⁻⁷	1	4	1	9	10	1/10 x100 = 10.0
10 ⁻⁴	0	5	0	14	14	0/14 x100 = 0
10 ⁻⁹	0	5	0	19	19	0/19 x 100 = 0
10 ⁻¹⁰	0	5	0	24	24	0/24 x 100 = 0

Table showing the percentage of Neutralization at various NDV Dilutions.

Neutralization Index = $\{\% \text{ Neutralized at dilution immediately above } 50\% - 50\%\} / \{\% \text{ Neutralized at dilution immediately above } 50\% - \% \text{ Neutralized at dilution immediately below } 50\%\}$

Dilution of 10^{-5} % Neutralized above 50% = 71.4%

Dilution of 10^{-6} % Neutralized below 50% = 37.5%

Neutralization index = $71.4\% - 50\% / 71.4\% - 37.5\% = 21.4/33.9 = 0.6$

The Neutralization index is then applied to the dilution that produced the percentage neutralized immediately above 50%, which is 10^{-5}

The index of 0.6 is applied to this dilution = $10^{-5-0.6}$ or 1 neutralized dose (ND₅₀) is $10^{-5.6}$

Therefore Neutralization titre = $10^{-5.6} \text{ ND}_{50} / 0.1 \text{ ml}$

DISCUSSION

The study showed that ASAP colloidal silver solution had a neutralizing effect on the Newcastle Disease Virus. This supports the conclusions made that the ASAP Colloidal Silver Solution is naturally antimicrobial (killing harmful bacteria, yeast, fungus, viruses). The results obtained revealed that the higher the dilutions, the greater the neutralizing effect of ASAP on NDV. All eggs were cut open and the allantoic fluid tested for neutralization activities by the Hemagglutination Inhibition test. From the table 1, results show that ASAP had 71.4% (above 50%) neutralization effect on NDV at the dilution of 10^{-5} and 37.5% at dilution of 10^{-6} . At dilution of 10^{-7} , ASAP had 10% neutralization effect on the Newcastle Virus. Therefore the researcher/alternate hypothesis, Ha, which states that ASAP has a neutralization effect on the Newcastle Disease Virus is accepted and the null hypothesis, which stated "ASAP has no neutralizing effect on the Newcastle Disease Virus and as such the neutralizing dose is not significant" is thus rejected. The dilution index of the ASAP solution is 0.6 and when applied to the dilution that produced the percentage neutralized immediately above 50% which is 10^{-5} , it resulted in a 1 neutralized dose of (ND₅₀) of $10^{-5.6}$. Therefore the Neutralization titre is $10^{-5.6} \text{ ND}_{50} / 0.1 \text{ ml}$. The control eggs were all intact and were all positive to NDV by the HA technique. This helped validate the experiment.

CONCLUSION /RECOMMENDATIONS

This study revealed that ASAP colloidal silver solution has neutralizing effects on Newcastle Disease Virus. This was proven from the results obtained after testing different dilutions of ASAP against NDV. The finding of this study confirms the conclusions made that the ASAP Colloidal Silver Solution is naturally antimicrobial (killing harmful bacteria, yeast, fungus, viruses) and that the ASAP has neutralizing effects on NDV. More work should be done to establish the antiviral/neutralization effects of ASAP on other level four viral pathogens such as the haemorrhagic fever viruses viz, Ebola, Marburg, Lassa fever virus e.t.c. It seems promising as a future candidate antiviral agent. The use of ASAP either as an immune booster or for the treatment of any kind of ailment and infections should be done under the prescription and supervision of a qualified medical and veterinary professional. Its level of toxicity (being a highly diluted solution of silver) was zero when critically appraised using laboratory animals.

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