Antiviral Effects, of a synthetic Aluminium-Magnesium Silicate, on *Avian Influenza Virus* .

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Summary.

Effects of a synthetic Aluminium-Magnesium Silicate [AMS] on *Avian Influenza Virus (AIV)* were tested. Equal amounts of H_5N_1 AIV *samples* and of AMS were mixed, left one hour, at room teperature before centrifuging. The supernatants were remeasured and tested for viral titre, for Mean Death Time (MDT) and Embryo Mortality Rate (EMR) of chicken eggs.

Volumes of the viral samples reduced at rate of 23.4 ± 5.48 %. Viral titres reduced significantly (P= 0.001) from HA, 73 ± 32.72 to 1.4 ±0.43 . Also, mortality of infected embryos reduced from 100 % to 65% while MDT of those that died,increased significantly (P = 0.001) from 76 ± 4.38 to 130 ±17.27 hours .When incubation with AMS was repeated on portions of same sample ,MDT increased from 64 to 104 hours with the portion incubated once .Two AIV portions on which incubation with AMS was repeated could not kill chick embryos.

Background.

Avian Influenza Viruses are enveloped, single stranded RNA viruses.Two different protein projections cover their envelopes. These are the Haemagglutinin (H) and the Nueraminidase (N) antigens. Combinations of the H and the N antigens is an important property of *Influenza viruses* because, it allows for resortment when two *Influenza viruses* replicate in same cell at the same time.

Influenza viruses are classified into types, A, B and C, based on antigenic differences of their neuclocapsid and of their matrix proteins[1].*Avian Influenza Viruses* are *Influenza A viruses* [2].

Influenza A viruses are subdivided serologically, into sixteen H and nine N subtypes. All the subtypes have been identified in avian species and they coexist with their natural hosts (water fowls and sea birds) in perfect harmoney [2]. Disease outbreaks occur in domestic birds when they come in contact with these carriers or with infected pigs [2]. Infected birds shed *Influenza viruses* in their secretions and excretions.So, domestic birds can also get infected when they contact secretions or excretions of infected wild birds or formites [3] .

Avian Influenza viruses cause two main forms of disease, distinguished by low or high virulence in domestic poultry. The low pathogenic form causes only ruffled feathers and drop in egg production

while the Highly Pathogenic *Avian Influenza Viruses* (HPAIV) spread rapidly between flocks , affect many organs of infected birds and lead to high mortality within 48 hours [3].

Genetic characterization of *Avian Influenza Virus* samples isolated from outbreaks in Nigeria, in 2007 revealed a new resortant virus (H_5N_1) [4]. Resortment occurs when an *Influenza virus* infects a human being or a pig that is already infected with another *Influenza virus*. Such resortment of antigens (viral shift),can produce *Influenza virus strains* , different from available vaccine strains. Such viral shift led to human pandemics of 1918 and of 1957 - 1958. So, isolation of a new *Influenza Virus* subtype in Nigeria is of great concern to the country, both for her poultry industry and for public health.

AMS is safe when ingested by man or animals . For this reason, it is used in many pharmaceutical formulations [5]. It is used in tableting drugs because, it`s molecules have one of their ends positively charged and the other, negatively charged [6]. Viruses have electrical charges too. Some have net positive electrical charges while others have net negative charges [7].

These electrical charges could make *Influenza Virus* particles adsorb onto molecules of AMS. If this happens, first stage of viral infection, which is adsorption to hosts` cells may be inhibited.

Natural AMS has impurities [6]. These could cause adverse reactions on treated animals if the natural ore is ingested at the high doses that may be required to treat viral diseases. So, two medicinal minerals which occur in Nigeria, Aluminium Silicate $\{Al_4(SiO_4)_3\}$ and Magnesium Silicate $\{Mg_2SiO_4\}$ were reacted to get a synthetic AMS : $Al_4(SiO_4)_3 + 3Mg_2SiO_4 \rightarrow 2Al_2Mg_3(SiO_4)_3$. [8]

Summary of Methods:

Samples of the H_5N_1 AIV**,** isolated from outbreaks of *Bird Flu* in Nigeria were incubated with the AMS . To a measured volume of each viral sample, equivalent amount of the synthetic AMS (on volume to weight basis) was added. They were thoroughly mixed and allowed to stand at room temperature for one hour before they were centrifuged at 3000 revolutions per minute for ten minutes. Volumes of their supernatants were remeasured and tested for viral titre by the haemagglutination method [9]. Also, 0.1ml of each supernatant was innoculated into yolk sac of two embryonated chicken eggs to determine Mean Death Time (MDT) and Embryo Mortality Rates (EMR) of infected chicken embryos. The eggs were candled every 12 hours to check for embryo mortality. Embryos that remained alive 208 hours post infection (PI), were recorded to have survived the infection with the AIV.

As controls, intact portions of each of the AIV samples were used for HA test along with supernatants of their portions incubated with the synthetic AMS, on same plates. Also, portions of each of the intact viruses were used for egg inoculation along with supernatants of their portions incubated with the AMS. Means of the viral titres and of the MDT of the two groups were used to test the null hypothesis that AMS has no effect on titre and pathogenicity of AIV, by the Student T – test (n =10).

Portions of a sample of the, H_5N_1 AIV which gave high HA titre were used to test effect of repeating incubation of AIV with AMS, on titre of the virus and on MDT and EMR of chicken embryos innoculated with it. One portion was incubated with the AMS once. To a second portion, after the centrifugation,

equal amount of the AMS was mixed with the supernatant and the incubation process was repeated once. In the third portion, incubation with the AMS was repeated twice. The three portions were then tested for viral titre and for MDT and EMR of chicken embryos innoculated with AIV.

Findings.

Volumes of the viral samples reduced at a mean rate of 23.4 ± 5.48 % following incubation with the AMS. Also, incubation with the AMS significantly (P = 0.001) reduced titre of the H_5N_1 AIV from a mean HA, 73 ±32.72 to 1.4 ± 0.43 and the EMR (P = 0.001) from 100 to 65 % .Incubating AIV with the AMS increased MDT of chicken embryos innoculated with the virus significantly (P = 0.002) from 76 ± 4.38 to 128.00 ± 18.36 hours.

Repeating incubation of AIV with the AMS reduced titres of the viral samples from HA, 128 to HA, 2 in both the portion incubated with AMS once and in the two portions incubated twice and thrice respectively. EMR of chicken embryos was 100% in the control and in the group innoculated with AIV portion incubated with AMS once, but their MDT increased from 64 in the control to 104 hours in the group innoculated with AIV portion incubated with the AMS once . In the group of eggs innoculated with portions of the AIV incubated with AMS twice and thrice respectively, there was no embryonic death,208 hours PI.

Effects the AMS had on volume of the viral samples, on the viral titres and on MDT and EMR of chicken embryos innoculated with the *Avian Influenza Virus* are as on tables 1, 2 and 3

Table 1: **Effect of incubating H_5N_1 *Avian Influenza Virus* with a Synthetic Aluminium – Magnesium Silicate on Volume of the viral samples.**

Viral samples	Volume before incubation With AMS (ml)	Volume after incubation with AMS (ml)	% Reduction
1	13	10	21.1
2	9	6.5	21.8
3	6	3.5	30
4	3	2.5	16.7
Mean	7.78± 4. 27	5.6± 3.38	23.4±5. 48

Incubating AIV with the AMS reduced volume of the viral samples significantly [P = 0. 001].

Table 2:**Effect of Aluminium – Magnesium Silicate on Titre of H_5N_1 *Avian Influenza Virus***

Virus samples	HA Titre	
	Control	Incubated with AMS
1	256	4
2	256	0
3	128	0
4	32	2
5	32	2
6	8	0
7	8	2
8	4	2
9	4	2
10	2	0
Mean	73±32.72	1.4 ± 0.43

Incubating AIV with AMS reduced the viral [HA] titre (P = 0.001).

Table 3: **Effect of Aluminium – Magnesium Silicate on Mean Death Time and on Embryo Mortality Rates of Chicken Eggs innoculated with H_5N_1 *Avian Influenza Virus.***

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Virus samples	MDT(Hours)		EMR(%)	
	Incubated with AMS	Control	Incubated with AMS	Control
1	112	88	100	100
2	52	88	100	100
3	76	40	100	100
4	64	88	100	100
5	148	76	50	100
6	148	76	100	100
7	136	76	100	100
8	208	76	0	100
9	208	76	0	100
10	118	76	0	100
Mean	128.00 ±18.36	76±4.38	65 ± 15.00	100 ± 0.00

Incubating AIV with AMS increased MDT (P = 0.002) and reduced EMR (P = 0.001) of Embryonated chicken eggs innoculated with the virus .

Discussion.

Brooks [10] defined antiviral agents as substances which kill viruses or substances which inhibit replication or pathogenicity of viruses. Stern *et al* [11] had reported that pathogenicity of myxoviruses recide in their haemagglutinin antigen. Since the synthetic Aluminium – Magnesium Silicate inhibited HA titre which is activity of the haemagglutinin antigen of H_5N_1, *Avian Influenza virus*, reduced the rate at which the virus killed chick embryos and delayed death even in the embryos that were killed , it suggests the AMS had antiviral effect against the virus. Reduction in volume of the viral samples suggests that the synthetic AMS *adsorbed* onto water molecules in the samples .This action of the synthetic AMS agrees with reported effect of natural AMS on water [5].Reduction in volume of viral samples should lead to increase in the viral titre if the AMS had no effect on the virus. However,the viral titres reduced inspite of the reduction in volume. This suggests that the electrostatic attraction

between the AMS molecules and the viral particles was more than that between them and water molecules.Reduction in titre of the HPAI virus and inhibition of the viral activities by AMS as seen in these experiments agree with earlier results of effect of the synthetic AMS on *Peste des Petits Ruminants Virus* [12], on *Infectious Bursal Disease* Virus [13] , on *Egg drop syndrome 76 virus* [14] , on *Canine Parvovirus* [15] and on *Newcastle Disease Virus* [16].

AMS is safe when ingested by man and by animals [5,17].It is used to bind drugs to make tablets used in treating both humans and animals [6]. Foster and Smith [18] reported that AMS has been in use for treatment of ulcer for many years. Also, the synthetic AMS tested in this study,was got by reaction of Aluminium Silicate and Magnesium Silicate [8] which are medicines being used to treat both animals and human beings. So,the synthetic AMS should be safe for treating viral diseases of man and of animals. It can be used for systemic treatment of infectious diseases,because,once there is inflamation of mucous membranes of the stomach or of the intestines even unabsorbable subatances can pass into the blood stream [19] . Also, simple sugars are reported to carry drugs accross intact mucous membranes by active transport [20]. Dextrose monohydrate incorporated in the AMS [8] would carry the AMS accross even uninflamed mucous membranes.Ability of the AMS to inhibit replication or pathogenicity of the HPAI virus as suggested by the significant increase in MDT and decrease in EMR recorded in these experiments shows that it could be a good candidate for development of a drug for control of Bird Flu in poultry and for management of human cases of Avian Influenza.

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