Available online at http://www.ajol.info/index.php/ijbcs

Int. J. Biol. Chem. Sci. 5(2): 835-839, April 2011

ISSN 1991-8631

International Journal of Biological and Chemical Sciences

Short Communication

http://indexmedicus.afro.who.int

Effects of Aluminium–Magnesium Silicate on *Newcastle Disease Virus* and on recovery of infected chicks

M. C. O. EZEIBE*, O. IJABO, C. UZOPUO, O. N. OKOROAFOR, J. I. EZE, I. J. MBUKO, M. E. SANDA, P. C. ANIMOKE and A. A. NGENE

Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.
**Corresponding author; E-mail: maduikeezeibe@yahoo.com*

ABSTRACT

Effect of a synthetic Aluminium – Magnesium Silicate (AMS) on *Newcastle Disease Virus* (NDV) was tested. Equal amounts, of the AMS and of NDV, on a volume to weight (v/w) basis, were incubated for one hour at room temperature, and then centrifuged. The supernatant was tested for NDV titre. Portions of a virulent NDV were similarly incubated with the AMS, and their supernatants tested for morbidity rates on infected chicks. Also, two groups of chicks were infected with virulent NDV intramuscularly (I/m), and by introduction of infected chicks, respectively. Their subgroups were treated by administering AMS through drinking water, to assess its effect on mortality. Incubating NDV with AMS, reduced the viral titre from mean HA, 613 ±86 to 4. 5 ±0.72 ($P < 0.05$). Also, incubating virulent NDV with the AMS, reduced its morbidity rate from 100% to 20% ($P< 0.05$) when incubated once, and from 100% to zero, when incubated twice. NDV- infected chicks treated with AMS, had same 100% mortality as the controls, when route of infection was I/m ($P > 0.05$) but when infection was by introduction of infected chicks, mortality reduced from 20% to zero ($P < 0.05$).These results suggest AMS as NDV's antiviral agent.

Keywords: Synthetic, Aluminium – Magnesium Silicate, Inhibition, *Newcastle Disease Virus*.

INTRODUCTION

Newcastle Disease (ND) is a very contagious viral disease of poultry. It affects many of the avian species, including, chickens, turkeys, geese, ducks, pheasants, guinea fowls, ostritches, emus and rhea (Jordan and Pathason, 1996). All ages and classes of these species are affected but young ones are more susceptible (Arnall and Keymer, 1995).

ND was recognized for the first time in Newcastle – on – tine, hence its name (Gordon, 1977). It has been reported in most parts of the world (Alexander, 1975, Spradbrow, 1988). The disease is caused by a virus, *Newcastle Disease Virus*, an RNA virus of the genus paramyxovirus (Gordon, 1977). Transmission of NDV occurs naturally through aerosol and by contact between healthy birds and sick ones or through formites (Jordan and Pathason, 1996).

Alexander (1996) reported that the incubation period of ND is four to five days when infection occurs naturally but when the virus was inoculated by intramuscular injection, the chicks were dead in 48 hours. He also

reported that natural infection of NDV leads to the respiratory form of ND while infection by intramuscular (I/m) or by intravenous (I/v) route leads to the nervous form of ND.

Severity of ND also depends on strain of the NDV (Gordon, 1977). Mortality of the highly virulent, velogenic strain, ranges from 10% to 100%. The milder mesogenic strain causes reduction in egg production in adults and low mortality in chicks while the lenthogenic strain is avirulent and does not produce clinical signs in infected birds.

There is at present, no effective treatment for ND (Adene, 2004). Prevention consists of eradication and biosecurity measures while control is by vaccination (Allexander, 1977).

Vaccination failure is now common in Nigeria. Recent studies suggest that most cases of vaccination failure in the country, are due to poor storage of vaccines because of frequent power outages. Rapid decline of already acquired immunity due to high environmental temperature is also being suggested as one of the causes of vaccination failures in Nigeria. There is therefore need to search for effective treatment for ND to reduce mortality in infected flocks.

Aluminium – Magnesium Silicate is a mineral which is said to occur naturally in India and in the United States of America (Jacina and Wordnslay, 2005). Vanderbilt (1995) reported that the natural AMS contains many impurities. However, when purified, AMS has been used in treating various diseases in humans and animals (Foster and Smith, 2007). Schils (2002) reported that the EEC committee on veterinary medicinal products has approved use of AMS for treatment of food animals.

AMS molecules possess two electrically charged ends. One end carries positive electrical charges while the other is negatively charged (Vanderbilt, 1995). Viruses also have electrical charges (Cann, 1993). So, AMS may have antiviral effect because extracellular viruses could adsorb to the AMS by electrostatic attraction. This may inhibit the first stage of viral infection which is adsorption to host cells.

To overcome the problem of impurities in the natural AMS, Aluminium Silicate and Magnesium Silicate which are certified medicinal minerals, were reacted to get a pure form of the AMS (Ezeibe, 2006).

Tests of the synthetic AMS on viruses have shown that it has antiviral effects on *Egg Drop Syndrome 76 virus* (Ezeibe et al., 2010a), on *Infectious Bursal Disease Virus* (Ezeibe et al., 2009b), and against *Peste des Petits Ruminants Virus* (Ezeibe et al., 2009c). Incubating a highly pathogenic Avian Influenza virus (HPAIV) with the AMS also reduced the viral HA titre and increased Mean Death Time of chick embryos inoculated with the HPAIV (Ezeibe et al., 2009a). Experiments were therefore designed to test antiviral effects of the AMS on NDV *in vitro* and *in vivo*.

MATERIALS AND METHODS

Field strains of Velogenic *Newcastle Disease* virus, isolated in Nigeria (Echeonwu et al., 1993), were used for the experiments. The Aluminium – Magnesium Silicate was synthesized as described by Ezeibe (2006).

Portions of NDV samples were mixed with the AMS on an equal volume for weight (v/w) basis. The mixtures were kept at room temperature for one hour and were centrifuged at the rate of 3000 revolutions per minute for ten minutes. In one experiment, incubation of NDV with AMS was repeated on the viral supernatant as already described.

HA test was performed with eight samples of NDV incubated with the AMS once. As control, portions of the eight NDV samples which were not incubated with the AMS were also used for HA test on the same plate as their portions incubated with the chemical.

For the test on effect of AMS on morbidity of NDV, three groups, each of 10, five weeks old chicks were used. In one

group, each chick was inoculated with 0. 2ml of a virulent NDV incubated with the AMS once, by intramuscular (I/m) route. In the second group, each chick was inoculated with 0.2 ml of the same NDV after it was incubated with the AMS twice, also by I/m route. In the control group, the chicks were similarly inoculated with same NDV sample without first incubating it with the AMS. The three groups of chicks were kept in different cages and were observed for clinical signs of ND.

To test effect of the AMS on mortality of NDV infected chicks, two groups, each of 20 chicks were used. One group was infected by injecting each chick 0.2 ml of the NDV, I/m. The second group was infected by introducing four chicks earlier infected with the NDV, into their pen, to mimic natural mode of infection. Following observation of clinical signs of ND, including anorexia, dullness, cough and diarrhoea, in the two groups, they were each subdivided into two of 10 chicks each. One of the subgroups was treated by administering AMS in their drinking water at the rate of 2 g per litre, for seven days. The second subgroup in each case served as untreated controls. Mortality rates were recorded for each of the four subgroups.

Mean HA titre of the NDV portions incubated with the AMS was compared with mean HA titre of their portions not incubated with AMS, by the 'students t- test', while the morbidity rates and mortality rates of the test groups were compared with their controls by Chi-square method (Steel and Torrie, 1960).

RESULTS

Incubating NDV samples with the AMS reduced the viral (HA) titre from a mean of 613 ± 86 to 4.5 ±0.72 ($P < 0.05$). Incubating NDV with the AMS once, also reduced its morbidity rate from 100% to 20% while incubating the virus with the AMS twice reduced the morbidity from 100% to zero. When chicks were infected by I/m inoculation of NDV, both the group treated with AMS and the control, had 100% mortality each ($P > 0.05$) but when infection was by mimicking the natural mode of infection, treating with AMS reduced mortality from 20% to zero ($P < 0.05$). HA titres of the NDV incubated with the AMS are as shown on Table 1.

Table 1: HA titres of samples of *Newcastle Diseases Virus* incubated with a synthetic Aluminium-Magnesium Silicate.

NDV samples	**Viral Titres (HA)**	
	Incubated with AMS	**Control**
1	8	128
2	4	2048
3	8	256
4	4	128
5	0	8
6	4	2048
7	4	32
8	4	256
Mean ± SD	**4.5 ± 0.72**	**613 ± 86**

Incubating NDV with AMS reduced the viral titre ($P < 0.05$).

DISCUSSION

Reduction in titre of NDV following incubation with the AMS and failure of portions of the virus incubated twice with the chemical, to reproduce ND in susceptible chicks infected by I/m inoculation, suggest that the AMS may have adsorbed to the NDV particles in the samples by electrostatic attraction between the electrical charges on its molecules and opposite charges on the viral particles and so the viruses were eliminated by centrifugation. Also, in the control group, clinical signs of ND were observed 40 hours post infection (P/I) but in the group inoculated with same NDV incubated with AMS only once, even the two chicks that became sick, showed clinical signs of ND, 5 days PI. This suggests that even with the single incubation with the AMS, NDV titres of the samples were drastically reduced. The low morbidity (20%) and prolonged incubation period in the group inoculated with NDV incubated with AMS only once, agrees with reduction in the titre got *in vitro*.

Alexander (1961) reported that when chicks are infected with NDV by natural mode (ingestion and/or inhalation), it leads to respiratory and enteric forms of the disease but, when infection is by intramuscular or intravenous route, nervous form of ND is produced. Nerves are poorly vascularised. So, most drugs do not reach pathogens in the nerves. It is possible that the AMS could not get to the NDV particles in the group infected by I/m inoculation of NDV, hence the 100% mortality in the treated subgroup. In the group infected by mimicking natural mode, the virus may have localized in the gastrointestinal tract (Echeonwu, 1993) and in the respiratory tract (Alexander, 1961). So, the AMS may have reached the virus and adsorbed onto the viral particles, hence the 100% recovery in the group's treated subgroup.

ND is recurrent in poultry populations in Nigeria, especially in birds kept in confinement (Echeonwu, 1993). Yet there is no drug available in the country to treat poultry infected with NDV (Adene, 2004). Since AMS is safe for use on food animals (Schils, 2002), it may be useful in reducing mortality of ND in naturally occurring epizootics and in cases of vaccination failures.

Every virus has genome and viral genomes are made of a number of positively charged ions such as Na^{+}, Mg^{++} and K^{+} while the phosphate component is negatively charged. Some viruses end up with net positive charges while others end up being negatively charged (Cann, 1993). Molecules of AMS have both positive and negative electrical charges. Possession of electrical charges by viruses and by AMS may be responsible for the antiviral effects which the synthetic AMS has shown against all four viral families so far tested, including *Othormyxoviridae* (Ezeibe et al., 2009a), *Paramyxoviridae* (Ezeibe et al., 2009b), *Birnaviridae* (Ezeibe et al., 2009c) and *Parvoviridae* (Ezeibe et al., 2010a; Ezeibe et al., 2010b). This suggests it has broad spectrum antiviral effect and may be effective against other viruses of animals and man.

The synthetic AMS is cheap and from results of *in vivo* experiments with chicks and dogs (Ezeibe et al., 2009c; Ezeibe et al., 2010b), it does not produce side effects when used to treat avian species and mammals. So, it may be a candidate for development of cheap, broad spectrum antiviral dugs for treatment of viral diseases of the avian species and of mammals.

REFERENCES

Adene DF. 2004. General stock management for disease prevention in poultry. In *Poultry Health and Production-Principles and Practices.* (1st edn). Stirling-Horden Publishers Nigeria.

Alexander DJ. 1996. Paramyxoviridae (Newcastle Disease and Others). In *Poultry Diseases* (4th edn). W. B. Saunders Co Ltd.

Alexander DJ. 1997. Newcastle disease and others paramyxovirus infections. In *Diseases of Poultry* (10th edn). Ames, Iowa State University.

Arnall L, Keymr IF. 1975. Bird Diseases: An introduction to the study of birds in health and disease.

Cann AJ. 1993. Principles of Molecular Virology (2nd edn). Academic Press: New York.

Echeonwu AG, Iroegora ON, Emeruwa CU. 1993. Recovery of velogenic Newcastle Disease Virus from dead and healthy free roaming birds in Nigeria. *Avian Pathology*, **22**(121): 383-387.

Ezeibe MCO. 2006. Admacine®. Federal Republic of Nigeria. Patents and Designs Act. Cap 344 LFN 1990 No. No 16446.

Ezeibe MCO, Egbuji AN, Eze JI, Ijabo O. Ngene AA, Okoroafor ON, Eze IC, Ugonabo JAC. 2009a. Effect of a synthesized Aluminium–Magnesium Silicate on *Avian Influenza* virus sub type H_5N_1. *Int. J. Phys. Sc.*, **1**(2): 73 – 76.

Ezeibe MCO, Mbuko IJ, Okoroafor ON, Okonkwo AC, Animoke PC, Orajaka LJE, Ngene AA. 2009b. *In vitro* and *in vivo* Effects of Aluminium-Magnesium Silicate on *Infectious Bursal Disease Virus* of chicken. *Anim. Sc. Rep*., **3**(4): 132-137.

Ezeibe MCO, Ijabo O, Okoroafor ON, Orajaka LJE, Ukomadu NM, Chukwu OS, Ngene AA. 2009c. Antiviral Effects of Aluminum-Magnesium Silicate on *Peste des Petits Ruminants Virus. Anim. Sc. Rep*. **3**(4): 141-147.

Ezeibe MCO, Okoroafor ON, Ijabo O. Ukomadu NM, Ngene AA, Eze JI. 2010a. Haemagglutination and Haemagglutination-Inhibition titres of *Egg Drop Syndrom 76 virus* treated with Aluminium – Magnesium Silicate. *Anim. Sc. Rep.*, **4**(3): 87 – 90.

Ezeibe MCO, Nwaogu IC, Nwigwe AN, OkoroaforON, Eze JI, Ngene AA. 2010b. Aluminium– Magnesium Silicate inhibits *parvovirus* and cures infected dogs. *Health*, **2**(10): 1215-1217.

Foster E, Smith K. 2007. Aluminium-Magnesium Silicate is used as antacids, antiulcer and control of diarrhoeas. *J. Ren. Nutri*., **4**(2): 50-53.

Gordon RF. 1977. Newcastle Disease. In *Poultry Disease*. The English Language Book Society. Baicilliere Tindal: London.

Jacyna S, Wormsley R. 2005. Aluminium-Magnesium Silicate used in drug formulation and cosmetic. *Bull. Mater. Sci.*, **24**(6): 377-382.

Jordan FTW, Pattson M. 1999. *Infectious Diseases of Poultry*. W.M. Saunders Co.

Schils S. 2002. The use of montmorillonite in the fight against harmful affects of ammonia. *J. Ren. Nutri*., **4**(2): 32-36.

Spradbrow PB. 1988. Newcastle Disease. In *Developments in Veterinary Virology*, Alexander DJ (ed). Kluwer Academic Publishers: Boston .

Steel RGD, Torrie JH. 1960. *Principles and Procedures of Statistics*. McGraw-Hill Book Co. Inc.: New York.

Vanderbilt RT. 1992. *Veegum the Versatile Ingredient for Pharmaceutical Formulation*. Inc. Technical Literature; 15-20.