INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560-8530/99/01-1/2-042-044

Effect of Physico-chemical Factors on Survival of Newcastle Disease Virus

RIZWAN QAYYUM, MUHAMMAD NAEEM AND KHUSHI MUHAMMAD
Microbiology Section, College of Veterinary Sciences, Lahore, Pakistan

ABSTRACT

Effect of physical (temperature, pH and ultraviolet light) and chemical (formalin, phenol crystal, iosan, aldekol, and bromosept) factors on survival of Newcastle disease virus was evaluated. It was observed that the virus endured 56°C for 30 minutes but got inactivated within 45 minutes. The virus remained active at pH 4 and 9 upto 24 hours but lost its viability at pH 1 and 13 within six hours. The virus remained active following exposure of ultraviolet light for 45 minutes. Amongst the chemical factors, formalin at 0.48% concentration inactivated the virus in 30 minutes, while its 0.24 and 0.12% concentration did not inactivate the virus in 45 minutes. Phenol crystal at 0.4% and aldekol at 0.5% concentration inactivated the virus within 15 and 45 minutes, respectively. Bromosept at 1 and 0.5% concentration inactivated the virus within 15 and 30 minutes, respectively. Iosan at both the concentrations i.e. 0.5 and 1% inactivated the virus within 15 minutes. It is concluded that virus can survive in the environment for long time while it can be inactivated on the farm premises by the chemicals.

Key Words: Physico-chemical factors; Survival; Newcastle Disease Virus

INTRODUCTION

Newcastle disease (Ranikhet; ND) is a highly contagious disease of poultry all over the world. It is causing heavy economic losses to the poultry industry in the form of high morbidity and mortality. Inspite of mass vaccination programmes and improved hygienic measures, it is still a common disease of poultry inviting attention of workers for its control. The disease is caused by a ND virus (NDV), a member of the Paramyxovirus-1 (Alexander, 1989). There are nine serotypes of paramyxoviruses i.e. PMV_1 to PMV_9. NDV is existing in the environment in three pathotypes i.e. velogenic, mesogenic and lentogenic (Calnek *et al.*, 1991). Transmission is by ingestion of infected feed or by inhalation of infected fomites. Windborne spread of ND has also been reported (Lancaster & Alexander, 1975). The virus can survive in poultry premises for 120 days (Jordan, 1990), and remains a source of infection for susceptible chicks in the vicinity. The present project was planned to evaluate the effect of physico-chemical factors on the survival of mesogenic strain of NDV. The results of this study helped in formulating biosecurity measures on the farms.

MATERIALS AND METHODS

Source of NDV. NDV was isolated from infected domestic birds and was characterised as mesogenic strain by Allan *et al.* (1978). The virus was cultivated in 11 days old chicken embryos. The allanto-amniotic fluid (AAF), harvested from such embryos was titrated on basis of its haemagglutination (HA) potential. The AAF was diluted upto 4 HA unit titre. Fresh chicken eggs were cleaned, incubated and candled. The eggs having live embryos were processed for evaluating the physico-chemical factors on the virus viability.

Treatment of NDV with physico-chemical agents. Nutrient broth (Difco) was prepared, autoclaved and incubated at 37°C for 24 hours for checking its sterility. The broth was divided into aliquot in sterilised test tubes (each containing 4 ml quantity). Each aliquot was mixed with equal volume of the AAF containing 4 HA unit of the virus. In this way, each aliquot finally contained 2 HA unit of the virus. Each broth with NDV suspension was exposed to 56°C , ultraviolet light, and different pH for different time intervals (Table I). The disinfectants used for inactivation of NDV included Formalin (Formaldehyde; Merck), Phenol cystal (Carbolic acid), Iosan (Iodine; Ciba-Geigy), Aldekol (combination of aldehydes, alcoholes & cationic biocides; EWABO Chemikaliem GmbH, Germany) and Bromosept was mixed with the AAF to achieve the required concentration. The virus-disinfectant mixture was incubated at 37°C for 15, 30 and 45 minutes.

Inactivation of NDV. Each of the virus suspension exposed to physical factors or disinfectants was inoculated in 11 days old chicken embryos (0.1 ml : allantoic route). The embryos were incubated at 37°C for 48 hours. The allantoic fluid was harvested from each of the embryo and tested for its HA activity (Senne, 1989). Lack of HA activity of the AAF indicated that physical

or chemical factor had inactivated the virus and vice versa.

RESULTS AND DISCUSSION

NDV proved to have high resistance to ambient temperature. However, it was inactivated at 56°C after 45 minutes of exposure (Table I). There is variation in thermostability of NDV. Therefore, quite controversial results from different parts of the world has been reported. For instance, Kim *et al.* (1978), Lomniczi (1975) and Hanson and Spalatin (1978) observed that the V_4 strain of NDV retained HA at 56°C for longer than 60 minutes. Likewise, Singh and Singh (1969) reported that 22 virulent virus strains retained their hemagglutinating activity when exposed to 56°C. As opposed to it, Khadzhiev and Hadjiev (1974) and Buxton and Fraser (1977) inactivated the NDV at 56°C within 45 minutes. The exact mechanism of heat mediated virus inactivation is not known. It is, however, expected that physical factors such as temperature are responsible for decreasing the polymerase activity of the virus which ultimately affects its replication activity (Stanwick & Hallum , 1976).

The UV light has no deleterious effect on the virus replicating ability (Table I) even after 45 minutes of exposure. These findings are in contrast to those reported by Sheaff *et al.* (1972) who inactivated the NDV using ultraviolet radiations. This variation could be due to the differences in virus exposure time to UV light and/or strain of the virus.

It was observed that the NDV strain lost its viability when exposed to pH 1 or 13 for 6-24 hours while it retained its virulence at pH 4 and 9 for similar period of time. Similar observations have been recorded by Sheaff *et al* (1972) and Hanson *et al.* (1967) who observed no detrimental effect of pH 3 on lentogenic strain of NDV; and who reported that at a pH 2.5, virus infectivity was not affected as found in the current study.

The results revealed that NDV can be inactivated by disinfectants at double of the recommended concentrations (Table III). NDV was inactivated with Formaldehyde (0.48% after 30 minutes), Iosan (0.5 & 1% after 15 minutes), Phenol crystal (0.4 & 0.6% after 15 minutes), Aldekol {0.5% (45 minutes) & 1%(30 minutes)} and Bromosept {0.5%(30 minutes) & 1%(15 minutes)}. The inactivation of NDV with disinfectants may be attributed to the possibility of their binding with ligand molecules which failed to adsorb on receptors of host cells. Similar observations regarding disinfectant-induced inactivation of NDV have been reported by Gale and Taylor (1947), Ismail *et al.* (1976) and Song and Lee (1988). However, the extent of the virus infectivity to be destroyed depends upon the strain of the virus, exposure time, quantity of the virus, nature of the medium and interaction between the treatments (Beard & Hanson, 1984).

Table I. Effect of temperature and ultraviolet light on the survival of Newcastle Disease Virus

Physical Factors (n=4)	Exposure Time (minutes)		
	15	30	45
Temperature (°C)	++++	++++	------
Ultraviolet Light	++++	++++	++++

Table II. Effect of pH on the survival of Newcastle Disease Virus

pH (n=4)	Exposure Time (hours)			
	06	12	18	24
01	-------	------	-------	-------
04	++++	++++	++++	++++
09	++++	++++	++++	++++
13	-------	------	-------	-------

Table III. Effect of chemical factors on the survival of Newcastle Disease Virus

Disinfectant	Concentr-ation (%)	Exposure Time (minutes)		
		15	30	45
Formalin	0.12	++++	++++	++++
	0.24	++++	++++	++++
	0.48	++++	-------	------
Iosan	0.5	-------	-------	-------
	1.0	-------	-------	-------
Phenol	0.2	++++	++++	++++
Crystal	0.4	-------	------	-------
	0.6	------	-------	-------
Aldekol	0.1	++++	++++	++++
	0.5	++++	++++	------
	1.0	++++	-------	------
Bromosept	0.1	++++		
	0.5	++++	-------	
	1.0	-------	-------	

++++ = AAF from four inoculated chicken embryos showed haemagglutination (HA) activity; ------ = AAF from four inoculated embryos showed undetectable HA activity.

CONCLUSIONS

The NDV can be inactivated in the poultry farms/hatcheries using high temperature (56°C), low (one) or high (13) pH of the material. It, however, is not practically feasible for the farmers. Use of disinfectants seems more appropriate and feasible. Therefore, there is no need to depopulate the poultry sheds for long time before arrival of newstock if disinfectants are used.

REFERENCES

Alexander, D.J., 1989. Newcastle disease. *In: Laboratory Manual for the Isolation and Identification of Avian Pathogens.* 3rd ed., Kendal/Hunt Publishing Company, USA. pp. 114-20.

Allan, W.H., J.E. Lancaster and B. Toth, 1978. Newcastle disease vaccines their production and use, FAO/UNO, Rome, pp. 57-62.

Beared C.W. and R.P. Hanson, 1984. Newcastle Disease. *In: Diseases of Poultry.* 8th ed., Iowa State University Press. Ames, Iowa, USA. pp. 452-70.

Buxton, A. and G. Fraser, 1977. *Animal Microbiology.* Blackwell Scientific Publications, Oxford. pp. 459-527.

Calnek, B.W., H.J. Barnes, C.W. Beard, W.M. Reid and H.W. Yoder, 1991. *"Diseases of Poultry"* 9th ed., Iowa State University Press, Ames. Iowa., pp. 496-513.

Gale, E.F. and E.S. Taylor, 1947. Action of fyrodicin and some detergent substances in releasing aminoacids from the internal environment of *Streptococcus faecalis. J. Gen. Microbiol.,* 1: 77-84.

Hanson, R.P., J. Spalatin, J. Estupian and G. Schloer, 1967. Identification of lentogenic strains of Newcastle disease virus. *Avian Dis.,* 11: 49-53.

Hanson, R.P.and J. Spalatin, 1978. Thermostability of haemagglutinin of Newcastle disease virus as a strain marker in epizootological studies. *Avian Dis.,* 22: 659-65.

Ismail, A.S., P.E. Woelfing and H. Geissler, 1976. Antiviral activity of some chemical disinfectants against some poultry pathogenic viruses. I. Newcastle disease virus (NDV). *Assiut. Vet. Med. J.,* 3: 215-29.

Jordan, F.T.W., 1990. Paramyoviridae (Newastle disease and others) *In: Poultry Diseases.* 3rd ed., Bailliere Tindall, London., pp: 121-36.

Khadzhiev, G. and G. Hadjiev, 1974. Heat and acid stability of the infectivity of Newcastle disease virus strains isolated in Bulgaria. *Veterinarnomeditsinski-Nauki, Bulgaria,* 11: 78-87.

Kim, S.J., P.B. Spradbrow and V.S. Chang, 1978. The serological response of chikens to Australian lentogenic strains of Newcastle disease virus. *Aust. Vet. J.,* 54: 430-6.

Lancaster, J.E. and D.J. Alexander, 1975. Newcastle disease virus and spread. Ottawa Department of Agriculture, Canada. pp. 79.

Lomniczi, B., 1975. Thermostability of Newcastle disease Virus strains of different virulence. *Archiv. Virol.,* 47: 249-55.

Senne, D.A., 1989. Virus propagation in embryonating eggs. *In: Laboratory Manual for the Isolation and Identification of Avian Pathogens.* 3rd. ed., Kendal/Hunt Publishing Company, USA. pp. 178-81.

Sheaff, E.T., A. Meager and D.C. Barke, 1972. Factors involved in the production of interferon by inactivated Newcastle disease virus. *J. Gen. Virol.,* 17: 163-75.

Singh S.B. and I.B. Singh, 1969. Some studies on the characterization and differentiation of Newcastle disease virus. *Indian Vet. J.,* 46: 1007-9.

Song, C.S. and T.C. Lee, 1988. Effects of chemical inactivants on viral polypeptides of Newcastle disease virus. *Res. Rep. Rural Develop.,* 30: 77-89.

Stanwich, T.H. and J.V. Hallum, 1976. Comparasion of RNA polymerase associated with Newcastle disease virus and temperature sensitive mutants of Newcastle disease virus. *J. Virol.,* 17: 68-73.

(Received 02 February 1999; Accepted 04 March 1999)