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Effect of Physico-chemical Factors on Survival of Avian Influenza Virus (H7N3 Type)

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ABSTRACT

Effect of physical (temperature, pH and ultraviolet light) and chemical (formalin, phenol crystal, iosan, Virkon-S, aldekol, Fourtedes, Kemstrong, Sanisquit and Superstop) factors on survival of avian influenza virus (H7N3 type) was evaluated. It was observed that the virus endured 56ºC for 60 minutes but got inactive at pH 1, 3, 10 and 14 within 48 hours while remained active following exposure to UV light for 45 minutes. Amongst the chemical factors inactivated the avian influenza virus at 0.5% concentration on 90 minutes post-interaction time. The technique can be used to evaluate the efficacy of disinfectants.

Key Words: Avian influenza; Virus; Inactivation; Survival

INTRODUCTION

Poultry industry has become dependent on the prophylactic measures of commercial birds during their life span, which has created a careless attitude towards management. Such management and poor bio-security result in an emergence of microbial diseases on the poultry farms. A severe out break of the avian influenza virus (AIV) was recorded during 1995 in all breeds of broiler breeder stocks (Hubbard, Indian River, Ross, Lohman and Arbor Acre), commercial layers and broilers (Bhatti, 1995). The disease inflicted heavy losses to the commercial poultry enterprises. The AIV (local virulent strain) was isolated from the organs of morbid birds (Muhammad *et al*., 1997). The AIV is secreted in the droppings of the clinically infected birds (Himshaw *et al*., 1979), which could be the possible source of infection to the surrounding poultry farms. Presently no outbreak of the AIV-H7 type has been reported, but the chances of reoccurrence of the malady are still there because vaccination against the AIV is not strictly practiced. This threat of the avian influenza has necessitated the extensive use of disinfectants, which are effective against wide range of viruses, bacteria and fungal spores. There is a wide variety of disinfectants available in market which are claimed to be effective against pathogens. The information about the efficacy of physical and the chemical (disinfectants) is scanty.

This project, therefore, was designed to evaluate the efficacy of various physical (temperature, Ultraviolet light and pH), and chemical (commercially available disinfectants) agents against local strain of AIV.

MATERIALS AND METHODS

Source of AIV. Local isolate of AIV-H7 type was procured from Avicenna Laboratories Limited, Faisalabad Road, Sheikhupura. The virus was propagated in 11 days old chicken embryos (allantoic route : 0.1 ml). On 48 hours post-inoculation, allanto-amniotic fluid (AAF) of these embryos was harvested and subjected to haemagglutination activity as described by Allan *et al*. (1978).

Physical factors. The AIV was diluted upto 4 HA units with peptone water (0.5 aqueous solution of peptone : pH 7.0) and exposed to various physical factors i.e. pH, ultraviolet light and temperature for different time periods. The AIV samples were collected after exposure of the AIV to the agents and stored at -20ºC until required.

Chemical agents. Different concentrations of disinfectants (formalin, phenol crystal, Iosan, virkon-S, Aldekol, Fourtedes, Kemstrong, Saniquid and Super Stop) were admixed with 4 HA diluted AIV and incubated for interaction for different time intervals. The samples were collected and inoculated in chicken embryos as described by Allan *et al*. (1978).

Inoculation in chicken embryos. Each of the samples was inoculated in each of the four chicken embryos (11 days old: donated by Avicenna Laboratories Limited). Four embryos were inoculated with untreated AIV suspension (4 HA titer) as control. The inoculated embryos were incubated at 37ºC and were candled after every 24 hours for consecutive 72 hours. All the dead or live embryos were chilled and the AAF harvested from each of the embryo was subjected to HA activity using 1% chicken erythrocytes. The inactivation of the virus by physical and chemical treatment was indicated by the survival of the embryo and lack of HA activity of the AAF. The results are displayed in form of age of embryonic death.

RESULTS AND DISCUSSION

Avian influenza is one of the devastating viral diseases of poultry with a tendency of rapid spread and inducing high morbidity (100%) and mortality (upto 80%). The casual agent of the disease is excreted in droppings of the diseased bird which results in the contamination of litter, feed, feeders, water, drinkers, air, eggs/egg trays, sheds and surroundings. The movement of the contaminated materials and persons from the infected farm disseminate virus to the other farms and susceptible birds in the vicinity (Narayan *et al*., 1969; Calnek *et*

al., 1991). The infectivity of the AIV is eliminated by the natural physical factors and chemical agents (Laver, 1963; Khafizova, 1976). It was observed that AIV was unable to retain its infectivity at pH of 1, 3, 10 and 14 while UV proved useless to kill AIV for a period of 45 min (Table I & II); while it retained its infectivity at 4, 30 and 37ºC for a period of 35 days. However, the HA activity was reduced to undetectable level.

Table I. Effect of pH on survival of the avian influenza virus

pH	Exposure time (hours)	Embryonic death (%)	HA activity in the AAF (n=5)
1	24	0	− − − − −
	48	0	− − − − −
3	24	0	− − − − −
	48	0	− − − − −
7	24	100	+ + + + +
	48	100	+ + + + +
10	24	0	− − − − −
	48	0	− − − − −
14	24	0	− − − − −
	48	0	− − − − −

+ + + + + = The allanto-amniotic fluid (AAF) from all of the five embryos inoculated with the treated avian influenza virus showed haemagglutinating (HA) activity; − − − − − = The AAF from all of the five embryos inoculated with the avian influenza virus did not show HA activity

Table II. Effect of ultraviolet light on survival of the avian influenza virus

Exposure time (minutes)	Embryonic death (%)	Effect on the virus (n=4)
15	50	+ + − −
30	75	+ + + −
45	75	+ + + −

+ + + + = The allanto-amniotic fluid (AAF) from all of the four embryos inoculated with the treated avian influenza virus showed haemagglutinating (HA) activity; − − − − = The AAF from all of the four embryos inoculated with the avian influenza virus did not show HA activity

AIV was able to withstand 56ºC for 45 min (Table III). These results are partially in line with the findings of Stallknechetal *et al*. (1990c) who reported that the virus can survive for 102 days at 28ºC and is inactivated at 60-70ºC within 10-20 min. The present study revealed that the natural physical factors are partially effective to inactivate the AIV. However, AIV can survive in the litter for long time (Stallknecht *et al*., 1990b).

AIVs are enveloped, relatively sensitive to inactivation by lipid solvents such as detergents. Ether and chloroform disrupt the flu virion by dissolving the lipid bilayer (Buxton & Fraser, 1977; Fenner *et al*., 1991). Infectivity is also rapidly destroyed by formalin, beta-propiolactone, oxidizing agents, dilute acids, ether, sodium deoxycholate, hydroxylamine, sodium dodecyl sulphate and ammonium ions (Laver, 1963; Franklin & Weeker, 1959). The virus interaction with formalin at concentration of 0.06 and 0.12% resulted in inactivation of the AIV within six hours. Formaldehyde is an economical and commonly used disinfectant in commercial poultry farms and is used to inactivate the virus for preparation of antigens and inactivated vaccines. The aldehyde (radical) part of the molecule binds with amine group of amino acid in proteins (Russel, 1990). Presently, it is deduced that formalin at 0.12% concentration for 12 hours at 37ºC inactivate the AIV in AAF suspension. However, formalin is carcinogenic in nature, therefore, beta propiolactone (BPL) is preferred to inactivate the virulent viruses for vaccine and antigen preparations. The phenol crystal solution is commonly used for sterilization of instruments and syringes at the poultry clinics/poultry farms. The phenol solution (0.2%) inactivated the virus (Table IV), but the aforesaid concentration of the phenol crystal is irritating to the living tissue but did not kill the embryos.

Table III. Effect of temperature (56ºC) on survival of the avian influenza virus

Exposure time (minutes)	Embryonic death (%)	HA activity in the AAF (n=5)
15	100	+ + + + +
30	100	+ + + + +
60	100	+ + + + +
90	0	− − − − −
120	0	− − − − −

+ + + + + = The allanto-amniotic fluid (AAF) from all of the five embryos inoculated with the treated avian influenza virus showed haemagglutinating (HA) activity; − − − − − = The AAF from all of the five embryos inoculated with the avian influenza virus did not show HA activity

Table IV. Effect of formalin and phenol on survival of avian influenza virus

Disinfectants	Conc. (%)	Interaction time (hours) n= 3			
		6	12	18	24
Formalin	0.06	+ − −	− − −	− − −	− − −
	0.12	+ − −	− − −	− − −	− − −
	0.24	− − −	− − −	− − −	− − −
Phenol crystals	0.1	+ + +	+ + +	+ + −	+ − −
	0.2	+ + −	+ + −	− − −	− − −
	0.4	+ − −	− − −	− − −	− − −

+ + + + += The allanto-amniotic fluid (AAF) from all of the three embryos inoculated with the treated avian influenza virus showed haemagglutinating (HA) activity; − − − − − = The AAF from all of the 3 embryos inoculated with the avian influenza virus did not show HA activity

The other disinfectants (commercial products) were found effective at double of their recommended concentration in 45 minutes. These disinfectants might be effective at half of the recommended concentrations when interaction time is increased to more than 45 minutes (Table V). It was further noted that disinfectant-virus interaction continues even during storage at -20ºC (Table VI). It must be born in mind that these disinfectants were mixed with peptone water along with the virus. This broth might have delayed or potentiated the effect of the disinfectants depending upon its chemical nature.

Table V. Effect of disinfectants on avian influenza virus

Disinfectants	Conc. (%)	Virus disinfectant interaction n+4 time (minutes) n=4			
		30	60	90	120
Iosan	0.10	+−−−(25)	−−−−(0)	−−−−(0)	−−−−(0)
	0.50	−−−−(0)	−−−−(0)	−−−−(0)	−−−−(0)
	1.00	−−−−(0)	−−−−(0)	−−−−(0)	−−−−(0)
Virkon-5	0.50	++−−(50)	+−−−(25)	−−−−(0)	−−−−(0)
	1.00	−−−−(0)	−−−−(0)	−−−−(0)	−−−−(0)
	2.00	−−−−(0)	−−−−(0)	−−−−(0)	−−−−(0)
Aldekol	0.25	+−−−(25)	−−−−(0)	−−−−(0)	−−−−(0)
	0.50	−−−−(0)	−−−−(0)	−−−−(0)	−−−−(0)
	1.00	−−−−(0)	−−−−(0)	−−−−(0)	−−−−(0)
Fourtedes	0.25	−−−−(0)	−−−−(0)	−−−−(0)	−−−−(0)
	050	−−−−(0)	−−−−(0)	−−−−(0)	−−−−(0)
	1.00	−−−−(0)	−−−−(0)	−−−−(0)	−−−−(0)
Remstrong	0.10	+−−−(25)	+−−−(25)	−−−−(0)	−−−−(0)
	0.50	−−−−(0)	−−−−(0)	−−−−(0)	−−−−(0)
	1.00	−−−−(0)	−−−−(0)	−−−−(0)	−−−−(0)
Sanisquid	0.10	++−−(50)	++−−(50)	−−−−(0)	−−−−(0)
	0.50	++−−(50)	+−−−(50)	−−−−(0)	−−−−(0)
	1.00	+−−−(25)	−−−−(0)	−−−−(0)	−−−−(0)
Superstop	0.10	+++−(75)	+++−(75)	+++−(75)	+++−(75)
	0.50	+++−(75)	+++−(75)	+++−(75)	+++−(75)
	1.00	+++−(75)	++−−(50)	++−−(50)	−−−−(0)

Figures in parenthesis indicate mortality of the embryos; +++++= The allanto-amniotic fluid (AAF) from all of the four embryos inoculated with the treated avian influenza virus showed haemagglutinating (HA) activity; −−−−−= The AAF from all of the four embryos inoculated with the avian influenza virus did not show HA activity

It is concluded that exposure of the virus to the environment of the farms (physical factors) and to the chemicals inactivate the virus on the farms

REFERENCES

Allan, W.H., J.E. Lancaster and B. Toth, 1978. *Newcastle Disease Vaccines: Their Production and Use.* FAO Anim. Prod. Hlth., Series-10, pp: 32–9, 57–62, 135–9.

Bhatti, B.M., 1995. A review of incidence of Avian influenza. *Disease Agro-Livestock News,* 1: 1–4.

Buxton A. and G. Fraser, 1977. *Animal Microbiology,* Vol. II, pp: 311–45. Blackwell Scientific Publications, Oxford, UK.

Calnek, B.W., H.J. Barnes, C.W. Beard, W.M. Retd and H.W. Yoder, 1991. *Diseases of Poultry,* pp: 532–51. Iowa State University Press, Ames, Iowa, USA.

Fenner, F., P.A. Bachmann, E.P.J. Gibbs, F.A. Marphy, M.J. Student, D.O. While (Eds.), 1991. *Veterinary Virology,* pp: 473–84. Academic Press, New York.

Hinshaw, V.S., R.G. Webster and B. Turner, 1979. Water born transmission of influenza A viruses. *Intervirol.,* 11: 66–8.

Khafizora, E.D., 1976. Disinfection of Surfaces and air of large poultry houses against avian influenza. *Problemy veterinoi sanitarii,* 54: 69–73.

Naryan, O., G. Lang, and J.B.T. Rouse, 1969a. A new influenza: A virus infection in turkeys. *Arch. Gesmate. Virusforsch,* 26: 149–65.

Russel, A.D., 1990. The effect of chemical and physical agents on microbes: Disinfectants and sterilization. *In:* Topley and Willsons, *"Principles of Bacteriology, Virology and Immunity,* pp: 72–103. Edward Arnold, A division of hodder and stronghton. London, Melhairn, Auckland.

Stallknecht, D.E., M.T. Kearney, M. Shane and P.J. Zwank, 1990c. Effects of pH, temperature and salinity on persistence of Avian influenza virus in water. *Avian Dis.,* 34: 412–8.

Stallknechtg, D.E., S.M. Shane, D.A. Seme, M.T. Kearney and P.J. Zwank, 1990b. Avian influenza viruses from migratory and resident ducks of Coastal Lowsiana. *Avian Dis.,* 34: 398–405.

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