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STABILITY OF LOCAL AND IMPORTED NDV VACCINE UNDER DIFFERENT STORAGE CONDITIONS

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ABSTRACT

The present study has been done to evaluate the efficacy of vaccine during manufacturing, storage and distribution under fluctuation of temperature as the vaccines are very susceptible to temperature. The results showed that New castle disease virus induce infections in developing embryos that lead to death of embryo with few exceptions. Further infection is more rapidly fatal when the inoculated embryos are young, when the quantity of virus introduce is large and when the required incubation temperature is above 37^{o}C. Comparison of table 4 and 5, and graph 4 indicate that no significant difference was found in the viral content (HA) of local and imported vaccines before and after inoculation, when analyzed through ANOVA and 5% level of significance.

Comparison of Table 6, 7 and 8 as well as Fig. 5 indicate that the end point of EID50 (local vaccine) decreases with the length of channel of distribution or we can say that these are inversely proportional to each other.

INTRODUCTION

Immunological products, especially vaccines have brought to mankind benefits arguably greater than those provided by any other pharmaceutical product (Hugo and Russel, 1992).

Vaccines against a dozen or more still prevalent infectious diseases provide immunity for million of human; poultry and various other group of animals every year ((Hugo and Russel, 1992).

Viral vaccines are suspension of virus grown in embryonated eggs, in suitable cell culture or on suitable tissue. They are frequently presented as freeze dried preparations (Henderson, 1981, 1983). The viability of the bioagent during the storage of the dried vaccine under conditions, determined by optimum storage temperature and time has been found satisfactory (Darydkin *et al.,* 2001).

During the manufacturing, storage and distribution, care should be taken not to allow any marked temperature fluctuation to occur because it can damage the antigenecity of vaccines. Therefore the storage conditions which ensures that vaccines are maintained within temperature range that assures their stability is the basic requirement of every vaccination campaign (Hakulinent *et al.,* 1986 and Sunematsu *et al.,* 1985).

New castle disease virus is an economically important pathogen of poultry that may cause clinical disease that ranges from a mild respiratory syndrome to a virulent form with high mortality, depending on an isolates pathotype (Berinstein *et al.,* 2001).

New castle disease vaccines are highly susceptible to heat and sunlight. So the care must be taken in storage and transportation to avoid the chances of damage. It should be kept at -20^{o}C. The studies had shown that; it can remain stable for 07 years, when stored at -20^{o}C (Mishra *et al.,* 2001).

Efficacy of live ND vaccine depends on the stability can be measured in terms of alteration in the ability to cause infection, to agglutinate the cells and to induce an immunogenic response. These abilities can be destroyed by various chemical and physical treatments like heat, light, U.V., x-rays, oxidation processes, pH changes and chemical compounds. It also depends on the exposure tissue, quantity of virus exposed; nature of suspension medium etc.

The present study has been done to evaluate the embryo Infective Dose 50%. All strains of Newcastle disease virus induce infection of chick embryos that lead, with few exceptions, to death of the embryo. The lentogenic strain induces the infections without death in embryos (Mishra *et al.,* 2001). Infection is more rapidly fatal when the inoculated embryos are young, when the quantity of virus introduce is large and when the required incubation temperature is above 37^{o}C (Van Kampen, 2001).

Lentogenic strain when injected into the yolk sac kill embryos in 48 hours (Van Kampen, 2001). The route of infection modifies the order in which tissue becomes infected and the rate at which the virus titre increases in them (Gould *et al.,* 2001).

MATERIAL AND METHODS

Ten fold dilution (10^{-10}) of the vaccine used. Add 1ml of vaccine to 9ml saline and this will give the dilution 10^{-1} and this has to continue upto l0^{-10} dilution.

Preparation of RBC:

Take the blood from four chicks of 2-6 weeks old by disposable syringe and transfer it into Alsevers solution. The pH is adjusted to 6.1 with newly prepared 10% solution of Citric Acid. The RBCs are washed three times by centrifugation in physiological saline. The final suspension can be stored at +4^{o}C for several days, according to the degree of contamination.

Method:

Forty Embryonating eggs incubated 9-11 days at 37^{o}C candled for embryo viability the edge of the air space margin and embryo sac marked with a pencil. All eggs are marked with the titration number. Groups of five eggs should be marked from 10^{-5} to 10^{-10}. For control it is useful to add 5-10 additional eggs to the titration system and inoculate them with the sterilize diluents.

Disinfect the marked region of eggs with iodine and leave it for drying, then drill 2 holes one at air sac and other near the embryo sac. The eggs are then inoculated with the different marked dilutions, using sterilize 1ml syringes. Inoculation must start from 10^{-10} – 10^{-5}. The control eggs must be inoculated last. All eggs are then sealed with paraffin wax and incubated at 37^{o}C.

The eggs must be candled daily, and any embryo dying within the 1st 24 hours should be discarded as non-specific mortality. The incubation should be continued for 5 days. All remaining eggs should be chilled over night at +4^{o}C and then examine for the presence of viral haemagglutinin.

A loop of amnioallantoic fluid from the eggs on test is mixed with a loop full of a 10% suspension of washed chicken RBCs on a white plate. Haemagglutination is observed in about 15 seconds.

Calculation for end point:

The end point can be calculated from the Spearman Karber method by the formula:

$$m = XK + \tfrac{1}{2}\ d - d\ \Sigma\ ri / n$$

where,

m = end point.
XK = the log value of the last time of titration.
d = the increment log —a ten fold increment is 101.
Σ ri = the sum of all eggs in the titration that were not infected.
N = the number of eggs inoculated per dilution.

RESULT AND DISCUSSION

Table 1, 2 and 3 indicate the specification of vaccines used in sterility test, ELD50 and HI test respectively. The specification includes number of doses, batch number, expiry date, point of collection, manufacturer name etc. These informations are very necessary to record because these are not only concerned with the legal aspects but also concerned with the potency of vaccine. These tables indicate that local vaccines do not contain any information about expiry date therefore we can say that they are violating the drug labeling and packaging rules 1986. While the rest of the things mentioned in Tables 1, 2 and 3 of local and imported vaccines meet with the standard.

Table 1

Specification of new castle disease vaccine used in sterility test

S. No.	Vaccine	Doses	Batch No.	Expiry Date	Manufacturer Name	Date of Collection
1.	Local	500	2--94	Not Specific	PRI	10/8/1999
2.	Imported	1000	37382	30-9-01	SOLVAY	10/8/1999

Table 4 and Fig.1 indicate the HA result of the reconstituted local vaccine (N.D. lasota) for the presence of viral content before and after inoculation. A (21) decrease was found after use in manufacturer, at 37°C, at 45°C used for ELD50 and at 45°C used in the determination of HI level. While remaining did not show any significant difference in HA activity (Gould *et al.,* 2001).

The HA result of the imported vaccine Table 5 and Fig. 2 indicate, A(2^1) decrease was found after use in distributor, at 37°C, at 45°C used for ELD50 and at 37°C, at 45°C used in determination of HI level. While remaining did not show any significant difference in HA activity. In another study of low virulence Newcastle disease virus isolates from poultry, it has been found that 30% of the isolates had a more the thermostable hemagglutin than the lentogenic reference strains B1 and Lasota or live vaccine derived from those strains. Whether those strains with a more thermostable hemagglutin are truly indigenous or whether they could have originated from vaccines used in the flocks was unknown (King, 1986).

Table 2
Specification of Newcastle disease vaccines used in embryo infective dose 50 percent (EID 50) end point

S. No.	Vaccine	Category	Doses	Batch No.	Expiry Date	Manufacturer Name	Date of Collection
1.	Local	Manufacturer	500	2--99	Not Specified	PRI	10/8/1999
2.	Local	Whole Seller	500	2--99	Not Specified	PRI	10/8/1999
3.	Local	Retailer	500	2--99	Not Specified	PRI	10/8/1999
4.	Imported	Distributor	1000	37382	30-9-01	SOLVAY	10/8/1999
5.	Imported	Retailer	1000	37382	30-9-01	SOLVAY	10/8/1999
6.	Imported	37°C (24 hours)	1000	37382	30-9-01	SOLVAY	10/8/1999
7.	Imported	45°C (24 hours)	1000	37382	30-9-01	SOLVAY	10/8/1999
8.	Local	37°C (24 hours)	500	2--99	Not Specified	PRI	10/8/1999
9.	Local	45°C (24 hours)	500	2--99	Not Specified	PRI	10/8/1999

1. Poultry Vaccine Production Center, Korangi, Karachi.
2. Solvay Animal Health Inc., Mendota heights, MN 55120 Mfg. in Charles City, IA 50616.

Table 3
Specification of Newcastle disease vaccines used in haemagglutination inhibition (HI) Test

S. No.	Vaccine	Category	Doses	Batch No.	Expiry Date	Manufacturer Name	Date of Collection
1.	Imported	TAP Water	1000	37382	30-9-01	SOLVAY	10/8/1999
2.	Local	TAP Water	500	2--99	Not Specified	PRI	10/8/1999
3	Imported	WELL Water	1000	37382	30-9-01	SOLVAY	10/8/1999
4.	Local	WELL Water	500	2--99	Not Specified	PRI	10/8/1999
5.	Imported	37°C (24 hours)	1000	37382	30-9-01	SOLVAY	10/8/1999
6.	Local	37°C (24 hours)	500	2--99	Not Specified	PRI	10/8/1999
7.	Local	45°C (24 hours)	500	2--99	Not specified	PRI	10/8/1999
8.	Imported	45°C (24 hours)	1000	37382	30-9-01	SOLVAY	10/8/1999

Table 4
Haemagglutination test of local vaccines before and after inoculation

S. No.	Category	Before Inoculation (Log 2)	After Inoculation (Log 2)
	Used in EID50		
1.	Vaccine provided by Manufacturer	10	9
2.	Vaccine provided by Wholesaler	9	9
3.	Vaccine provided by Retailer	9	9
4.	37°C (24 hours)	9	8
5.	45°C (24 hours)	9	8
	Used in HI Test		
6.	37°C (24 hours)	9	9
7.	45°C (24 hours)	9	8
8.	Well Water	9	9
9.	Tap Water	9	9

$\overline{X}$ = 9.11, $\overline{X}$ = 8.66, S.D. = 0.32, S.D. = 0.49
1. Embryo infective done 50%
2. Haemagglutination inhibition test.

Table 5
Haemagglutination test of imported vaccines before and after inoculation

S. No.	Category	Before Inoculation (Log 2)	After Inoculation (Log 2)
	Used in EID50		
1.	Vaccine provided by Distributor	10	9
2.	Vaccine provided by Retailer	9	9
3.	37°C (24 hours)	9	8
4.	45°C (24 hours)	9	8
	Used in HI Test		
5.	37°C (24 hours)	9	9
6.	45°C (24 hours)	9	8
7.	Tap Water	9	9
8.	Well Water	9	9

$\overline{X}$ = 9.12, $\overline{X}$ = 8.37, S.D. = 0.35, S.D. = 0.5

Approximately 100,00 virus infected units are equal to 1 haemagglutinating unit. The number of viral particle in a preparation may be approximated on the basis of its haemagglutinating activity. However the haemagglutinating activity of the virus is not necessarily inactivated at the same rate as is the infectivity of the virus preparation. Further it is recommended by F.A.O. 1978 (WHO, 1989) that the estimate of haemagglutinating be confined to serological work connected

with the HI test, or to tests for the specificity of the viral preparation. The reason of this recommendation is that the haemagglutinin reaction estimates only the red cell agglutinating power of the sample. This reaction may be due to virus that is not active, or to viral subunits. In either case, the haemagglutinin reaction is not a reliable estimate of the amount of viable virus contained in a sample of vaccine.

Table 6
Embryo infective dose 50% manufacturer (Local)

Dilution (Log 10)	Titration Result					Injected (I)	Healthy (H)
	1	2	3	4	5		
-5	I	I	I	I	I	5 out of 5	0
-6	I	I	I	I	H	4 out of 5	1
-7	I	I	I	I	H	4 out of 5	1
-8	H	H	H	H	H	0 out of 5	5
-9	-	H	H	H	H	0 out of 5	5
-10	-	H	H	H	H	0 out of 5	5

m = XK + 1/2 d – d ri/n, m = ?, XK = 10, d = 1, n = 5, ri = 17
m = 10 + 1/2(1) – (1) x 17/5, m = 7.1 , Vm = 0.08, Sm = 0.28

Table 7
Embryo infective dose 50% of locally manufactured vaccine purchased from wholesaler

Dilution (Log 10)	Titration Result					Injected (I)	Healthy (H)
	1	2	3	4	5		
-5	I	I	I	I	I	5 out of 5	0
-6	I	I	I	I	I	5 out of 5	0
-7	I	I	I	H	H	2 out of 5	3
-8	H	H	H	H	H	0 out of 5	5
-9	H	H	H	H	H	0 out of 5	5
-10	H	H	H	H	H	0 out of 5	5

m = 6.9, EID50 = 10^6.9, Vm = 0.06, Sm = 0.24

Table 8
Embryo infective dose 50% of locally manufactured vaccine purchased through retailer

Dilution (Log 10)	Titration Result					Injected (I)	Healthy (H)
	1	2	3	4	5		
-5	I	I	I	I	I	5 out of 5	0
-6	I	I	I	I	I	5 out of 5	0
-7	I	H	H	H	H	1 out of 5	3
-8	H	H	H	H	H	0 out of 5	5
-9	H	H	H	H	H	0 out of 5	5
-10	H	H	H	H	H	0 out of 5	5

m = 6.7, EID50 = 10^6.7, Vm = 0.04, Sm = 0.2

Table 6 and Fig. 3 indicate the result of embryo infective dose 50% out of 6 dilutions, at 10^{5} all 5 eggs exhibited haemagglutination, at 10^{-5} all 5 produce HA, at 10^{-7} 3 eggs exhibited HA. While remaining did not agglutinate the RBCs. No agglutination occurred at 10^{-8}, 10^{-9} and 10^{-10} dilution. The end point for ELD50 in case of locally manufactured vaccine was found to be 7.1 with a variance 0.08 and standard error 0.28.

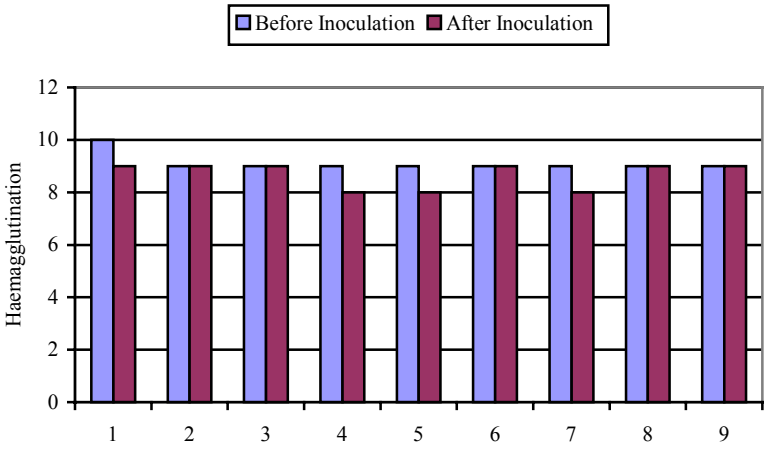

Fig. 1: Haemaggulation result of local vaccines before and after inoculation

1 = Collected from manufacturer
2 = Collected from wholesaler
3 = Collected from retailer
4 = Collected from stored at 37°C for 24 hours
5 = Stored at 45°C for 24 hours
6 = Stored at 37°C for 24 hours
7 = Stored at 45°C for 24 hours
8 = When applied with well water
9 = When applied with tap water

Fig. 2: Haemaggulation result of imported vaccines before and after inoculation

1 = Collected from distributor
2 = Collected from retailer
3 = Stored at 37°C for 24 hours
4 = Stored at 45°C for 24 hours
5 = Stored at 37°C for 24 hours
6 = Stored at 45°C for 24 hours
7 = When applied with tap water
8 = When applied with well water

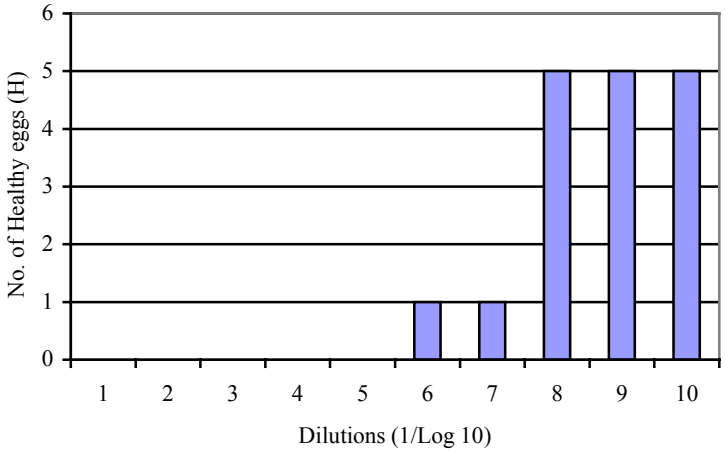

Fig. 3: Embryo infective dose 50%. When local vaccine was collected from manufacturer.

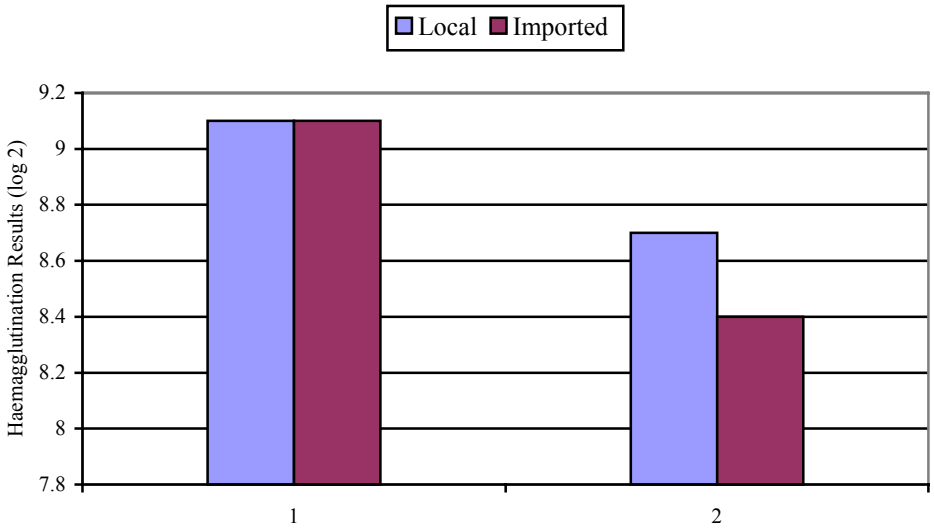

Fig. 4: Comparison of haemagglutination test result of vaccines before and after inoculation.
1 = Before inoculation 2 = After inoculation

The result is not in confirmation with standard FAO 1978 (WHO, 1989).

With lentogenic vaccine, the optimum range on the dose response scale is about $10^{7.0}$ EID50 per bird. This optimum range will vary with the vaccine under test, but is the standard recommended. By using the Spearman Karber technique for the end point detection a more accurate assay may be made on the viral content of batches of vaccine and the small loss in viability associated with freeze drying. The experimental error of a titration is a combination of the chance pattern of death in the inoculated eggs, the precision with which the operator carries out the test and accuracy of the pipette. Thus as indicated by Allan and Hebert (1968) it is not possible

to quote expected error of means. These authors gave result based on the titration by three different operators according to the methods outline above. The standard error of mean for ten fold dilution series was about +0 3 duplicate titration increase the accuracy of the estimation.

In general, on freezing virus loss will be greater, and in order to attain the optimal immune response the vaccine vials should be filled with 2m1 of allantoic fluid for each 1000 doses.

It is essential that vaccine retain a satisfactory potency during the storage period of 1 year. To determine this potency, an accelerated stability test should be carried out.

In general, storage of freeze dried vaccine at 37^{o}C for 12 days may be taken as equivalent to storage at -20^{o}C for 1 year. Alternatively, storage of freeze dried vaccine at 37^{o}C for 3 to 5 days may be equivalent to storage at +4^{o}C for 1 year.

Other results indicate that 1 week storage at 37^{0}C was equivalent to more than 2 years storage at +4^{o}C.

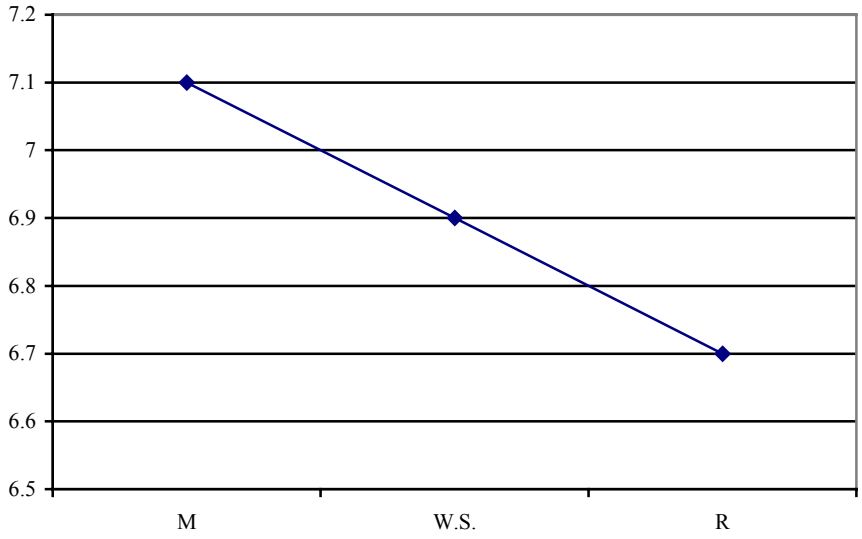

Fig. 5: Correlation between end point of embryo infective dose 50% and the channel of distribution.
M = Vaccine provided by manufacturer
W.S. = Vaccine provided by Wholesaler
R = Vaccine provided by retailer

STATISTICAL EVALUATION OF RESULTS

Conclusion:

Comparison of Tables 4 and 5, and Fig. 4 indicate that no significant difference was found in the viral content (HA) of local and imported vaccines before and after inoculation, when analyzed through ANOVA and 5% level of significance.

Comparison of Tables 6, 7 and 8 as well as Fig. 5 indicate that the end point of EID50 (local

vaccine) decreases with the length of channel of distribution. Or we can say that these are inversely proportional to each other.

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