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STUDIES FOR PREPARATION OF WATER STABILIZER FOR LIVE POULTRY VACCINE ADMINISTERED BY DRINKING WATER

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SUMMARY

Two vaccine stabilizers were prepared and evaluated for their effectiveness on the potency of Newcastle disease live vaccine (La Sota) administered in drinking water. The active ingredient in stabilizer 1 was Sodium Thiosulfate at final concentration of 0.014% in drinking water, and in stabilizer 2 was Sodium Thiosulfate and Polyethylene Glycol at final concentration of 0.014% and 0.01% in drinking water respectively. The virus titer in distilled water (D.W.), (D.W. + Stabilizer 1) and (D.W + stabilizer 2) were (6.7, 6.6 & 6.5), (6.7, 6.6 & 6.6) and (6.7, 6.6 & 6.5) log 10 EID_{50} at 0.0, 1.0 and 2.0 hours post incubation at room temperature. The virus titer in simulated tap water (STW) contained 4 ppm chlorine was declined from 6.3 to 1.2 log_{10} EID_{50} in the first hour post incubation and reached 0.0 log_{10} EID_{50} at 2 hours post incubation. Whereas the virus titer in (STW + Stabilizer 1) and (STW + Stabilizer 2) were (6.3, 5.2 and 4.8) and (6.3, 5.2 and 5.2) log_{10} EID_{50} at 0.0, 1.0 and 2.0 hours post incubation at room temperature respectively.

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INTRODACTION

Newcastle disease virus (NDV) is an economically important avian virus that is responsible for substantial loss to the poultry industry worldwide. Newcastle disease virus is classified as a member of the order *Mononegavirales*, family *Paramyxoviridae*, subfamily *Paramyxovirinae* **(Alexander, 2000)**. Vaccination with live and inactivated ND vaccines is considered the first line of defense protocol to prevent and control ND outbreaks. However, most commercially

available ND live vaccines are sensitive to adverse environmental conditions and required cold chain facilities which considered limiting factors for controlling ND **(Spradbrow, 1992)**. The route of administration of live ND vaccines poses obvious challenge for effective vaccination. while, eye drop, spray, and intramuscular routes stimulate better immune response than the drinking water route **(Ratanasethakul and Cumming,1983 and Branton *et al*., 2005).** But, the later has the advantages of easier management, not laborious and cost effectiveness. So, mass vaccination against ND through drinking water was early developed and has become a routine procedure for most poultry flocks **(Luginbuhi et al., 1955)**. Newcastle disease vaccination failure may be exist if drinking water contained antiviral agents, usually chlorine based, which inactivated the live virus vaccine **(Woodward and Tudor, 1975 and Bermudez and Brown, 2003)**. Therefore, there is an urgent need for vaccines to be stabilized to ensure their protection during drinking water vaccination process. Many substances were used as vaccine stabilizers, one of the earlier used vaccine stabilizers was skimmed milk which reduced the activity of both chlorine and quaternary ammonium based disinfectants **(Gentry and Braune, 1972)**.

Buffering agents such as Sodium bicarbonates, Sodium Citrate and Phosphate Buffer Saline were shown to protect live vaccines in drinking water by stabilizing the water (p^{H}) between 6 and 7 **(Leigh et al., 2008)**. Reducing agents such as Sodium Thiosulfate, Ammonium Thiosulfate, Sodium Bisulfite and Sodium metabisulfite were used for neutralizing free chlorine in drinking water **(Simpon, 2001)**. Thermal protective agents such as bovine serum albumin, sorbitol, maltose, lactose, sucrose and glycerol were also used as vaccine stabilizers **(Barbour et al., 2002)**.

Considering the steady increase of poultry farming, the water quality used in drinking water vaccination is difficult to assure and may causing vaccination failure. So, the objective of the present study is to prepare a vaccine stabilizer and evaluate its effectiveness to protect Newcastle disease live virus vaccines in drinking water.

MATERIALS AND METHODS

Vaccines

Live Newcastle disease vaccine (LaSota strain). It was locally prepared in Newcastle Disease Dept., Vet. Serum and Vaccine Research Institute, Cairo, Egypt.

Chemicals

Sodium Thiosulfate.
Polyethylene Glycol, (PEG).
Sodium hypochlorite (12%).
Imported commercial drinking water vaccine stabilizer (Aviblue).

Embryonated chicken eggs

Specific Pathogen Free (SPF) embryonated chicken eggs were obtained from Nile SPF Eggs, Koom - Oshiem, Fayoum, Egypt., to be used for virus titration.

Preparation of vaccine stabilizer

Two formulations of vaccine stabilizer stock solutions were prepared as follows:

-Stabilizer 1 (S1): 7 gm Sodium Thioslufate was dissolved in 10 ml distilled water.

-Stabilizer 2 (S2): 7 gm Sodium Thiosulfate + 5 gm PEG were dissolved in 10 ml distilled water.

800 mg of Neutral red dye was added to each of the two prepared stock solutions. The prepared stock solutions were distributed as 2 ml in glass vials, and freeze-dried to form lyophilized disc.The prepared stabilizers were formulated to serve 100 liter of drinking water for each.

Experimental design

Two experiments were conducted as follows:

The first experiment:

1. Three diluents each of 100 ml for vaccine mixing were prepared as follow:

- D1. Distilled water (DW).
- D2. Distilled water + Stabilizer 1 (DW+S1).

The lyophilized disc of stabilizer 1 was reconstituted in 10 ml distilled water, from which 10 µl was mixed in 100 ml distilled water.

- D3. Distilled water + Stabilizer2 (DW+S2).

The (S2) lyophilized disc was reconstituted in 10 ml distilled water, from which 10 µl was mixed in 100 ml distilled water.

2. The diluents stabilizer mixtures were incubated for 10 minutes at room temperature.

3. The lyophilized vaccine (LaSota) was reconstituted in 1.0 ml distilled water, from which 100 µl was mixed with each of the three diluents (D1-D3).

4. The three virus diluents mixture was incubated for two hours at room temperature.

5. Titration of vaccine virus in each of the three diluents was conducted in specific pathogen free embryonated chicken eggs at 0.0, 1.0 and 2.0 hours post incubation, the virus titer was calculated by method described by **(Reed and Muench, 1938)**.

The second experiment:

1. Four diluents each of 100 ml for vaccine mixing were prepared as follow:

- D4. Simulated tap water (STW).

It was made by mixing of Sodium Hypochlorite to D.W. at a final concentration of 4ppm.

- D5. Simulated tap water + S1, (STW + S1).

The (S1) lyophilized disc was reconstituted in 10 ml distilled water, from which 10 µl was mixed in 100 ml STW.

- D6. Simulated tap water + S2, (STW + S2).

The (S2) lyophilized disc was reconstituted in 10 ml distilled water, from which 10 µl was mixed in 100 ml STW.

- D7. Simulated tap water + Aviblue.

The imported drinking water vaccine stabilizer was mixed with STW at final concentration as recommended by the manufacturer.

2. The diluent stabilizer mixtures were incubated for 10 minutes at room temperature

3. 100 µl from the diluted vaccine virus (1/10) was mixed with each of the four diluents (D4-D7).

4. The four virus diluents mixtures were incubated for two hours at room temperature.

5. Titration of the vaccine virus in each of the four diluents was conducted as in experiment 1.

RESULTS AND DISCUSSION

In the absence of stabilizers, vaccines administered in water are likely to be inactivated by sanitizers' especially free chlorine. So, Maintenance of viable vaccine in drinking water is requisite for effective immune stimulation of vaccinated birds. From many stabilizing agent used as active ingredient in commercial vaccine stabilizer products (Table1), It is seemed to be nice to use Sodium Thiosulfate and Poly Ethylene Glycol in the preparation of the target vaccine stabilizers. Sodium Thiosulfate has a high reducing activity against chlorine based disinfectants, Moreover; it has the advantage over other reducing agents because it is recognized as a safe food additive **(Simpson, 2001).** Ploy Ethylene Glycol (PEG) act as good delivery system for vaccines administered through mucosal routes and enhance eliciting a high and long lasting immune response **(Vila *et al.*, 2004 and Anne des Rieux *et al.*, 2006)**.

The physical characters of the prepared vaccine stabilizers as shown in table (2) were acceptable in shape (disc form) which is convenient in management and facilitate accurate dosing (one disc / 100 Liter) without need for any additional calibrating equipment during vaccination process. The prepared vaccine stabilizers were proved to be easily dissolved in water without any remaining precipitates. Good dissolving property of the prepared vaccine stabilizers is of great importance and making them superior compared to skimmed milk which became limited in use because it is not convenient and cause blocking of nipple drinkers by non-dissolved residues which provide suitable media for bacterial growth in water pipes **(Cargill, 1999)**. Pink color of the prepared vaccine stabilizers facilitate easily monitoring of vaccine consumption by vaccinated birds through coloring its peak and tongue and assure the effectiveness of vaccination process.

The first experiment was conducted to ensure that the prepared vaccine stabilizers did not have any hazard to the vaccine virus itself, the effect of prepared vaccine stabilizers on the vaccine was compared to the

effect of distilled water alone. The obtained results (Table 3), showed that, the virus titer in distilled water (D.W), (DW + S1) and (D.W + S2) were (6.7, 6.6 & 6.5), (6.7, 6.6 & 6.6) and (6.7, 6.6 & 6.5) log $_{10}$ EID_{50} at 0.0, 1.0 and 2.0 hours post incubation respectively, indicating that both of the prepared stabilizers active ingredients (Sodium Thiosulfate and Polyethylene Glycol) have no detrimental effect on the virus viability up to two hours which is the maximum vaccination time recommended by poultry vaccines manufacturers for live poultry vaccine administered in drinking water **(Marangon and Busani, 2006)**.

Second experiment was conducted to evaluate the protective capacity of the prepared stabilizers to secure the vaccine virus from the determinate effect of chlorine when the vaccine rehydrated in simulated tap water (STW), and compare it with imported vaccine stabilizer. Chlorine veridical activity was previously documented at concentration of 2.0 and 2.6 ppm in drinking water **(Jordan and Nassar, 1973 and McDonnell and Russell, 1999)**. In the present study the available chlorine in prepared (STW) was adjusted to 4 ppm, which is considered a high content of chlorine in tap water to assure the protective ability of the prepared vaccine stabilizers under worst condition **(Al-Mayah et al., 2009)**.

The virus titer in simulated tap water (STW) contained 4 ppm chlorine was declined from 6.3 to 1.2 log_{10} EID_{50} / ml in the first hour post incubation and became 0.0 EID_{50} / ml at 2 hours post incubation (Table 4, Fig.1). The sever reduction of virus titer in chlorinated water was surprising and exceed our expectation when compared with result obtained by **(Kamau et al., 2010)** who reported slight reduction in Infectious Bronchitis live virus vaccine titers in water containing the same chlorine concentration of 4 ppm after 2 hours of incubation, which declined from 7.34 to 6.7 log $_{10}$ EID $_{50}$ / ml. The virus titer in D 5 (STW + S 1), D 6 (STW + S 2), and D 7 (STW + Imported stabilizer) were (6.3, 5.2 & 4.8), (6.3, 5.2 & 5.2) and (6.3, 5.0 & 5.0) at 0.0, 1.0 and 2.0 hours of incubation at room temperature respectively (table 4), which revealed that, both the prepared vaccine stabilizer as well as the imported one, greatly protected the live virus in chlorinated water.

The obtained results of the present study revealed that, Sodium Thiosulfate final concentration in chlorinated water is correlated to its protective capacity for live virus at different chlorine concentration. Whereas, **Jordan and Nassar (1973)**, reported incomplete protection for Infectious bronchitis virus titer which declined from 3.5 to 1.6 $\log_{10}$ EID_{50} (45 % protective capacity) within two hours in the presence of 0.01 % w/v Sodium Thiosulfate in water contain chlorine at 2ppm. Higher concentration of Sodium Thiosulfate (0.014 % w/v) used in this study alone or in combination with 0.01% w/v Polyethylene Glycol successfully preserved and protected the viability of live LaSota vaccine (76.0 – 82.5 % protective capacity) reconstituted in simulating tap water containing a higher concentration of chlorine 4 ppm for up to two hours.

Table (1) Active ingredients of some commercial drinking water vaccine stabilizers.

Product (Trade name)	**Active ingredients**
Aquamix	**Sodium Bicarbonate**, Milk Protein product **Polyethylene Glycol**, Citric Acid Sodium Croscarmellose and Artificial coloring
Aviblue	**Sodium Thiosulfate**, Citric Acid **Polyethylene Glycol** and Sodium Carbonate
Blufarm	**Sodium Thiosulfate** Patent Blue
BluPol	**Sodium Thiosulfate** Vitamin C.
Vactora Plus	**Sodium Thiosulfate**
Pre Vac	Prelevo glutamide, Vit. C Potassium Chloride Sodium Di acid Carbonate Skimmed milk powder
Vac. Guard	Animal Protein product Artificial coloring

Table (2) Physical characteristics of locally prepared vaccine stabilizer.

Item	Character
Form	Disc
Color	Pink
Odor	Odorless
Solubility	Highly soluble in water
Precipitate	No precipitates or residues observed

Table (3) Virus titer post rehydration in diluents without chlorine at time intervals.

Diluents		NDV titer/ Hours		
		0.0	1.0	2.0
D1	Distilled water (DW).	6.7	6.6	6.5
D2	Distilled water + Stabilizer1 (DW+S1).	6.7	6.6	6.6
D3	Distilled water + Stabilizer2 (DW+S2).	6.7	6.6	6.5

* NDV titer expressed as $\log_{10}$ EID_{50} /ml

Table (4) ND Virus titer post rehydration indifferent diluents at time intervals

Diluents		Time/ Hours					
		0.0		1.0		2.0	
		Virus titer	Survival (%)	Virus titer	Survival (%)	Virus titer	Survival (%)
D4	Simulated tap water (STW).	6.3	100 %	1.2	19 %	0.0	0.0 %
D5	Simulated tap water + S1 (STW + S1).	6.3	100 %	5.2	82.5%	4.8	76 %
D6	Simulated tap water + S2 (STW + S2).	6.3	100 %	5.2	82.5%	5.2	82.5%
D7	Simulated tap water + Imported stabilizer.	6.3	100 %	5.0	79 %	5.0	79 %

* NDV titer expressed as $\log_{10}$ EID_{50} /ml

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دراسات لتحضير مثبتات للقاحات الدواجن الحية التي تعطى في ماء الشرب

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الملخص العربي

تم تحضير عدد (٢) مثبت لقاح وتقييم كفاءتهما في حماية فيروس النيوكاسل (لاسوتا) في ماء الشرب. المادة الفعالة في مثبت اللقاح الأول هي (Sodium Thiosulfate) بتركيز نهائي في ماء الشرب ٠،٠١٤% وفي مثبت اللقاح الثاني (Sodium Poly Ethylene Glycol + Thiosulfate) بتركيز نهائي في ماء الشرب ٠،٠١٤% و ٠،٠١% على التوالي. جاءت عياريه اللقاح في الماء المقطر، (الماء المقطر + مثبت اللقاح الأول) و (الماء المقطر + مثبت اللقاح الثاني) (٦،٧، ٦،٦ و ٦،٥)، (٦،٧، ٦،٦ و ٦،٦) و (٦،٧، ٦،٦ و ٦،٦) لوغاريتم ١٠عند ٠،٠، ١،٠ و ٢،٠ ساعة من التحضين في درجة حرارة الغرفة على التوالي. انخفضت عياريه اللقاح في ماء مماثل لماء الصنبور يحتوى على كلور بتركيز ٤ جزء بالمليون من $١٠^{٦،٧}$ إلى $١٠^{١،٢}$ بعد ساعة من التحضين في درجة حرارة الغرفة ثم وصلت إلى ٠،٠ بعد ساعتين من التحضين في درجة حرارة الغرفة. بينما كانت عيارية اللقاح في (الماء المماثل لماء الصنبور + مثبت اللقاح الأول) و (الماء المماثل لماء الصنبور + مثبت اللقاح الثاني) (٦،٣، ٥،٢ و ٤،٨)، (٦،٣، ٥،٢ و ٥،٢) لوغاريتم ١٠عند ٠،٠، ١،٠ و ٢،٠ ساعة من التحضين في درجة حرارة الغرفة على التوالي.