KEEPING QUALITY OF LOCALLY PREPARED INFECTIOUS CORYZA VACCINE

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Abstract

Locally prepared aluminium hydroxide gel and oil adjuvant infectious coryza vaccines were stored at 4°C and at room temperature for 27 months after preparation. The potency of these vaccines was determined by immunization of chicken at different periods of storage. The immunizing power of aluminium hydroxide gel and oil adjuvant vaccines stored at room temprature decreased gradually after 2 months. At the same time, the potency of the same vaccines stored at 4°C was still protective till 12 months of storage for aluminium hydroxide gel vaccine and two years for oil adjuvant vaccine.

INTRODUCTION

Infectious coryza is a worldwide disease of the upper respiratory tract of chicken caused by *Haemophilus paragallinarum* (Yamamoto, 1984). The major economic effect of infectious coryza is an increasing culling rate in broilers, and reduction in egg production (10% -40%) in layers, as well as, reduction of quality and hatchability of eggs, (Taylor *et al.*, 1953) and (Yamamoto, 1984). In Egypt, infectious coryza was reported by (Eissa, 1960) and Farid *et al.*, 1966). In recent years, bacterins have been widely used to immunize chicken against infestious coryza (Clark and Godfrey, 1961, Page *et al.*, 1963 and Blackall *et al.*, 1992). Early vaccines were prepared in chicken embryos containing aluminium hydroxide gel or mineral oil as adjuvants (Matsumoto and Yamamoto, 1975, Davis *et al.*, 1976 and Coetzee *et al.*, 1983). In Egypt Zaki (1985), Ali (1987), Shafai (1991) and El-Sebaey (1996) prepared different monovalent and bivalent infectious coryza vaccines potentiated with aluminium hydroxide gel and oil as adjuvants for immunizing chickens aginst infectious coryza. Keeping quality and effect of storage temperature of infectious coryza vaccine was not done by any of the previous Egyptian researchers. The Anaerobic Research Department in Veterinary Serum and Vaccine Research Institute in Cairo started the production of oil adjuvant infectious coryza vaccine in 1994 and the vaccine labeled for one-year expiry date and must be stored at 4°C.

So, this work was done to determine exactly the duration of the most suitable storage coditions that maintain the efficacy and potency of locally prepared infectious coryza vaccine.

MATERIALS AND METHODS

I. Vaccines strains

W strain (serovar A) and Modesto strain (serovar C) were obtained from Intervet International B.V. Boxmer, Holland to use them in preparing *H.paragallinarum* vaccines. Two types of bivalent *H.paragallinarum* vaccines were used: aluminium hydroxide gel vaccine which was prepared according to Matsumoto and Yamamoto (1975), and oil adjuvant vaccine which was prepared according to Stone *et al.* (1987). The vaccines were bottled in 500 ml bottles, half numbers of these bottles were put in refrigerator at $4^{o}C$, and the other half were kept at room temperature.

2. Potency of prepared vaccines

The potency of each vaccine was determined just after preparation and 1, 2,3 months and then, every 3 months till 27 months after preparation. This was carried out by inoculation of each type of vaccine in groups of broiler chickens (20 birds for each group, 4-6 weeks old) which were bacteriologically tested to be free from *Mycoplasma, Pasteurella* and *H.paragallinarum* infections, and serologically to be negative for antibodies of *H.paragallinarum*. Each bird in the group received two doses of 0.5 ml each, one month apart, subcutaneously in the back of the neck (Blackall *et al.*, 1992). At the same time, 20 chickens were kept as a control (non-vaccinated control). Fourteen days after the second dose of each vaccine, blood sera were collected from all vaccinated and nonvaccinated chickens for serological test (tube agglutination test, according to Matsue *et al.*, 1978). At the same time, all birds were challenged by inoculation of 0.2 ml of 16 hours broth cultures of both W and Modesto virulent strains of *H.paragallinarum* by infraorbital sinus inoculation at a dose of $2x10^{7}$ CFU for each strain per bird (Blackall *et al.*, 1992). All chickens were examined daily after challenge for chinical signs of coryza for 10 days, and the trials, of isolation of *H.paragallinarum* were done to know whether it is the only causative agent for the clinical symptoms.

RESULTS AND DISCUSSION

Various workers have attempted to reduce the economic losses associated

with infectious coryza by the use of inactivated vaccines. Safe and effective adjuvants were used to enhance the immunogenicity of these vaccines. The results of studying the effect of temperature and duration of storage on the potency of locally prepared infectious coryza vaccine in terms of agglutinating mean titer are presented in Table 1 and Figure 1. Amongst various storage temperatures and periods, the refrigeration temperature of $4^{o}C$ storage gave the best results showing a maximum agglutinating titer of 256 from one day after manufacture till 3 months for oil adjuvant vaccine, and till 2 months for aluminium hydroxide gel vaccine, and decline in titers was observed till the end of the experiment. The same trend was observed for the storage temperature of $37^{o}C$ where the maximum agglutinating titer of 256 was recorded on the first day post-manufacture, and decline in titer was observed till 9 months for both oil adjuvant and aluminium hydroxide gel vaccines. These findings are parallel with those of Nisar (1988) who found that the storage temperature of $37^{o}C$ for various periods was unable to keep the vaccine potent. Matsumoto and Yamamoto (1975) mentioned that the chickens having agglutination titer of 1/32 were found to be protected against challenge exposure with *Haemophilus paragallinarum*. Statistical analysis using T test (Berly and lindgren, 1990) (Table 1) cleared that there was a significant difference between antibody titers in sera of chickens vaccinated with bivalent *H.paragallinarum* vaccine stored at $4^{o}C$ and those stored at room temperature from the ninth month post-vaccination till the end of experiment. On the other hand, there is no significant difference in antibody titer in sera of chickens vaccinated with the same vaccine stored at the same temperature from the first month post-vaccination till 6 months.

The results in Table 2 and Figure 2 show the potency of the vaccine for various storage temperatures and periods based on challenge infection of chickens. Oil adjuvant vaccine stored at $4^{o}C$ for 90 days remained potent and afforded 100% protection to vaccinated animals, then, the potency decreased to 80% after storage for 21 months or more. Aluminium hydroxide gel vaccine remained potent when stored at $4^{o}C$ for 60 days and gave 100% protection for challenged birds; the potency decreased to 80% after 12 months of storage. These findings are in partial agreement with those of Chandrasekaran *et al.*, (1987) who reported that, under refrigeration ($4^{o}C$) the potency dropped to 2.94%, 2.94%, 32.35%, 35.29% and 38.24% after 3,6,9,12 and 15 months storage, respectively. Also, Nangia *et al.* (1966) recorded that *Pasteuralla multocida* oil adiuvant vaccine was fully antigenic when stored at $7^{o}C$ for 814 days. For vaccines stored at $37^{o}C$, the potency dropped to 25%, 40% and 60% for oil adjuvant vaccine and 30%, 50% and 60% for alumin-

ium hydroxide gel vaccine after 6,12 and 15 months storage, respectively (Table 2 and Figure 2). These findings disagreed with those of Chandrasekaran *et al.* (1987), who found that the drop in potecy of vaccine stored in the cabinet ($29^{o}C$ - $32^{o}C$) was 5.88, 11.76 and 41.18 after storage for 3,6 and 9 months, respectively. Likewise, Vipulasiri *et al.* (1982) reported 43% and 86% drop in the potency of haemorrhagic septicaemia oil adjuvant vaccine after 6 and 12 months of storage at room temperature. The drop in potency of a vaccine is attributed to the loss in its stability (*Vipulasiri et al.*, 1982), or, to denaturation of the antigen (Chandrasekaran *et al.*, 1987). The data obtained from measuring the immune reponse using challeng test (Table 2) revealed that, there is no significant difference in immune response in animals inoculated with vaccine stored at $4^{o}C$ and those at room temperature from the first month post-vaccination till 15 months. On the other hand, there is significant difference in immune response of chickens vaccinated with the same vaccine stored at the same temperature from 18 month till the end of experiment.

It is evident from the results obtained in the present work that, infectious coryza vaccine is best stored at $4^{o}C$ and can be used safely and effectively up to 12 months for aluminium hydroxide gel vaccine and up to 2 years for oil adjuvant vaccine.

Table 1. Mean agglutinating titer in sera of chicken vaccinated with infectious coryza vaccine stored at different temperatures.

Period of storage	Mean agglutinating titer of chicken vaccinated with				Control chickens*
	Oil adjuvant vaccine		Alum.hydroxide gel adjuvant vaccine		
	Stored at 4°C	Stored at room Temperature	Stored at 4°C	Stored at room Temperature	
1 day	256	256	256	256	0
1 month	256	256	256	128	0
2 month	256	128	256	128	0
3 month	256	128	128	96	0
6 month	128	96	96	64	0
9 month	128**	48	96**	32	0
12 months	96**	16	48**	8	0
15 months	64**	4	32**	4	0
18 months	64**	2	16**	0	0
21 months	32**	0	8**	0	0
24 months	32**	0	4**	0	0
27 months	16**	0	4**	0	0

* No antibody titer in sera of non-vaccinated control chickens.

** Significant difference between vaccine stored at room temp. and that stored at 4°C.

Table 2. Potency of infectious coryza vaccines stored at different temperatures determined by challenge test.

Period of storage	Protection % of chicken vaccinated with				Control chickens*
	Oil adjuvant vaccine		Alum.hydroxide gel adjuvant vaccine		
	Stored at 4°C	Stored at room Temperature	Stored at 4°C	Stored at room Temperature	
1 day	100	100	100	100	0
1 month	100	100	100	90	0
2 month	100	90	100	80	0
3 month	100	90	90	80	0
6 month	95	75	85	70	0
9 month	90	70	85	65	0
12 months	90	60	80	50	0
15 months	85	40	60	40	0
18 months	85**	10	60**	10	0
21 months	80**	0	50**	0	0
24 months	80**	0	40**	0	0
27 months	70**	0	40**	0	0

* Non vaccinated control chicken show typical signs of infectious coryza.

** Significant difference between vaccie stored at room temp and that stored at 4°C.

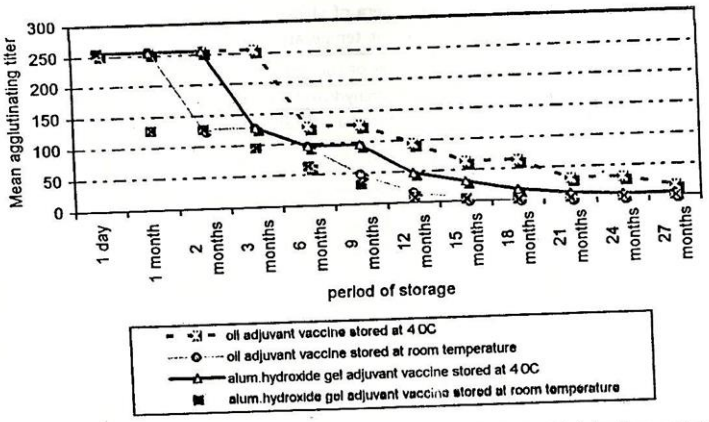

Fig. 1. Mean agglutinating titer in sera of chicken vaccinated with infectious coryza vaccine stored at different temperatures.

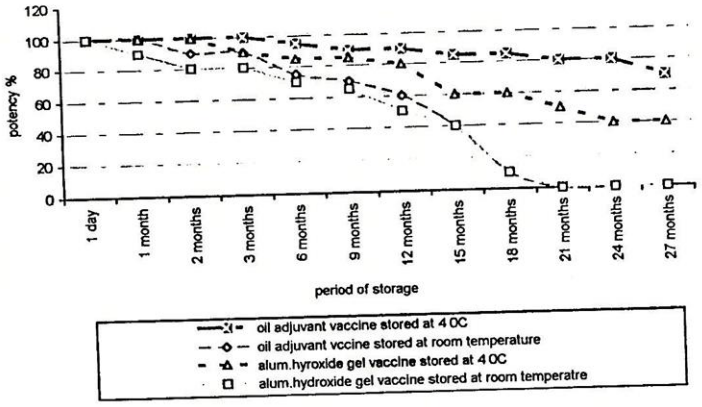

Fig. 2. Potency of infectious coryza vaccines stored at different temperatures determined by challenge test.

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كفاءة حفظ لقاح زكام الطيور المعدي المحضر محليا

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تم تحضير لقاح محلي من زكام الطيور وذلك باستخدام نوعين مختلفين من المحسنات (هيدروكسيد الألومنيوم جيل/والزيوت المعدنية). تم حفظ اللقاحين في درجة حرارة الثلاجة العادية (٤ - ٨ م) وأيضاً في درجة حرارة الغرفة وذلك لمدة سبعة وعشرين شهراً. قيست القوة المناعية لكلا اللقاحين علي فترات متباعدة وذلك بتحصين الدجاج باللقاحين محل التجربة.

وقد أثبتت النتائج أن كلا النوعين من اللقاح والمحفوظ في درجة حرارة الغرفة تناقصت قوته المناعية بعد مضي تسعة شهور من التخزين، بينما اللقاح المحضر باستخدام هيدروكسيد الألومنيوم جيل والمحفوظ في درجة ٤° م احتفظ بقوته المناعية حتي إثني عشر شهراً بخلاف اللقاح الزيتي الذي تم حفظه في الثلاجة عند درجة ٤ ° م فقد احتفظ بقوته المناعية حتي سنتين (٢٤ شهراً).