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THERMOSTABILITY AND KEEPING QUALITY OF OIL ADJUVANT FOWL CHOLERA VACCINE

(With 3 Tables)

By

SUSAN F. GORGI; S.M. ABOUL SAOUD and S.M. GERGIS

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دراسة تأثير التخزين ودرجات حرارة الحفظ المختلفه على الكفاءه المناعيه للقاح كوليرا الدواجن المثبط المطور

سوزان جورجى ، سيد أبو السعود
ساهر جرجس

عند دراسة انسب درجات التخزين التى تحافظ على ثبات وكفاءة لقاح كوليرا الطيور المطور وجد أن الدفعات الثلاثه التى تم تخزينها عند درجة ٤ درجه منويه أو ٢٨ - ٣٠ درجه منويه (درجة حرارة الغرفه) لمدة ٣٠، ٦٠، ٩٠، يوم أعطت اجسام مناعيه بعد التحصين بخمسة عشر يوما وأن الوسط الهندسى للاجسام المناعيه الخاصه لميكروب الباستيرلامالتوسيدا وصلت الى أقصى ارتفاع عند ٤٥ يوم بعد التحصين. وقد وجد أن الاجسام المناعيه الخاصه لميكروب الباستيرلا مالتوسيدا كانت أقل فى الدفعات التى تم تخزينها عند حرارة ٢٨ - ٣٠ ° م عن تلك التى تم تخزينها عند درجة حرارة ٤ ° م خاصة عند ٣٠، ٤٥ يوم بعد التحصين وقد تأكدت هذه النتائج باستخدام اختبار تحدى المناعه فى الدجاج والفتران المحصنه اذا كانت نسبة الحمايه مقبول فى الدجاج المحصن بواسطة اللقاح المخزون عند درجة ٤ ° م عن الدجاج المحصن باللقاح المخزون عند ٢٨ - ٣٠ ° م. وقد تبين أيضاً من الدراسه ان اللقاح كان قادراً على احداث تغير فى مستوى الاجسام المناعيه كما كان له تأثير ونسبة حمايه ٩٠٪ عند التخزين لمدة ٦ شهور واستمر قادراً على احداث الحمايه _ (٦٥٪) الى ١٥ شهر من التخزين. وبفحص الخواص الطبيعيه ونقاوة الدفعات الثلاثه للقاح السابقه وجد أنه ثابت ونقى حتى ١٥ شهر من تاريخ تصنيع اللقاح. ومما سبق وجد أن لقاح كوليرا المطور فى الدجاج يمكن استخدامه بكفاءه وصلاحيه حتى ١٥ شهر بعد التصنيع عند تخزينه فى درجة حرارة ٤ ° م.

SUMMARY

By studying the most suitable storange conditions that maintains the efficacy and stability of improved polyvalent fowl cholera vaccine for poultry, it is

evident that all the three batches that stored at 4 °C or 28-30 °C after 30, 60, or 90 days of storage start to induce a systemic humoral antibodies after fifteen days postvaccination. The geometric mean titres (GMT) of specific anti *Pasteurella multocida* antibodies increase till reach a maximum GMT on the 45th postvaccination day. There is a marked decreases of GMT in speccific anti *Pasteurella multocida* antibodies in batches that stored at 28-30°C than those stored at 4°C especially at 30th and 45th day postvaccination. These results is further documented by potency testing in chicken and mice as acceptable protection was noted in chicken vaccinated with avain cholera vaccine stored at 4°C and 28-30°C (at room temperature). The vaccine can induce seroconversion and evoke a protection of 90% at the 6th month of srorage. The protection still gave acceptable protection (65%) at the 15th month of storage. Regarding the physical properties and sterility of examined batches, it was obvious that all batches remaind stable and sterile up to 15 months after the date of manufaeture. In conclusion, the improved polyvalent fowl cholera vaccine for poultry can be used safely and effectively up to 15 months post manufacture date when stored at 4 °C.

Key words *: Fowl Cholera Vaccine Keeping Quality*

INTRODUCTION

Fowl cholera, caused by *Pasteurella multocida*, is one of the oldest known infectious dieasese of poultry, and is still constitute a major disease problem of chicken, ducks, and turkeys. Outbreaks of this disease can have special economic importance for large commercial chicken, duck and turkey breeders due to the rapidity of spread and the extraordinary virulence shown by many strains, Soliman (1983). Vaccination is acknowledged internationally as the best way of controlling fowl cholera disease, Gergis et al (1991).

In Egypt, Killed oil adjuvant bacterin of *Pasteurella multocida* are used as immunoprophylactic agents against fowl cholera in chicken, Genidy et al (1971) and in turkey, Zaher et al (1974). Consequently, numerous attempts have been made by researchers to identify the predominant isolated *Pasteurella multocida* strains that causing fowl cholera in chicken, Gergis (1978), in ducks, Ghanem (1986) and Gorgi (1992), and in turkeys, Fahmy (1992) to improve fowl cholera vaccine by adding these new isolated strains to the vaccine to be more efficient. In the last few years, researchers

succeded in improving fowl cholera vaccine by adding the new serotypes and changing some component of the adjuvant in the vaccine, Azzam et al (1992), but non of those researchers delt with the keeping quality and effect of storage temperature on fowl cholera vaccine.

This study was undertaken to investigate the most suitable storage conditions and duration of storage that maintains the efficacy, potency and stability of the improved polyvalent fowl cholera vaccine for poultry.

MATERIALS and METHODS

1- Chickens: three hundred and seventy chickens, six weeks old at the time of first vaccination, in groups (Ten in each group) were vaccinated with the recommended dose (0.5 ml) and the recommended route (subcutaneously) by the various lots of avian cholera vaccine. Booster vaccination was conducted 4 weeks after primary vaccination. Seven control unvaccinated Groups of chicken were included in each group. They were free from all infectious diseases and had neither a history of fowl cholera nor immunization with fowl cholera vaccine.

2- Mice: Eighteen groups of mice (fifty in each group) were also vaccinated with the various lots of avian cholera vaccine kept at different temperatures of storage, the room temperature and refrigerator and three groups were used as control unvaccinated groups.

3- Vaccine: three different batches (lots A, B, and C) of locally produced avian cholera vaccine obtained from Veterinary Serum and Vaccine Research Institute, abbassia, Cairo and subjected to thermostability and keeping quality testing were used. This vaccine was prepared from four serovars of *Pasteurella multocida (5:A, 8:A, 9:A, 2:D)*, that were most commonly encountered among poultry infected with fowl cholera in Egypt, was used. The details of vaccine preparation was previously described by Gergis et al (1991).

4- Physical properties: Various physical properties included dispersion in cold water, viscosity, separation through centrifugation at 400xg and standing at room temperature were observed.

5- Sterility test: All batches examined for sterility by growing on blood agar, nutrient agar, MaConkey's agar and incubation aerobically at 37 °C for 72 hours. Sabouraud agar was cultivated by the examined batches and incubated at room temperature for 14 days. Anaerobic cultivation was carried out on thrioglycolate media.

6- Thermostability test: Two bottles of each Lot. of avian cholera was stored at 4°C and 28-30 °C for studying, thermostability. Two groups of chicken (ten in each group) were vaccinated subcutaneously with the recommended dose (0.5 ml) of vaccine samples stored at the aforementioned temperatures after 30, 60, 90 days of storage. Booster vaccination was conducted 4 weeks after primary vaccination and challenge infection 50 days after the first vaccination.

7- Keeping quality test: To find out the keeping quality of avian cholera vaccine, three batches (A-C) of the vaccine were stored at 4 °C. After 6, 9, 12, and 15 months of storage at this temperature groups of chickens (ten each) were vaccinated with the recommended vaccination dose, route and scheme. Challenge infection was done 50 days after first vaccination.

8- Challenge infection: All vaccinated as well as non vaccinated chicken were challenged by the inoculation of 0.1 ml of 100 LD_{50} of virulent *Pasteurella multocida* strains used in the manufacture of the vaccine. Challenge infection was conducted 50 days after first vaccination. Inoculated chicken were observed for 10 days and mortalities were recorded.

Vaccinated and control mice were inoculated with different dilutions (from 10^{4} to 10^{-10}) of virulent *Pasteurella multocida* strains according to the method of Ose and Muenster (1968).

9- Experimental design for groups of chicken used for evaluation of 3 batches of fowl cholera stored at 4 °C and room temperature:

Intervals of storage	Vaccines stored at 4°C			Vaccines stored at 28-30°C			Control
	A	B	C	A	B	C	
	Batches			Batches			One
Thirty days(One month)	One	One	One	One	One	One	One
Sixty days(Two months)	One	One	One	One	One	One	One
Ninety days(Three months)	One	One	One	One	One	One	One
Six months	One	One	One	-	-	-	One
Nine months	One	One	One	-	-	-	One
Twelve months	One	One	One	-	-	-	One
Fifteen months	One	One	One	-	-	-	One

* Chicken groups (10 each).

10- Potency testing: At least 65% protection must be achieved in chicken for judjing a vaccine Lot. as valid. Concering mice 2 Log. protection must be achieved for judjing a vaccine Lot. as effective.

11- Serological testing: Anti *Pasteurella multocida antibodies* were measured by the passive haemagglutination test using the method of Carter and Rappy (1962).

A vaccine lot is concluded effective if it induce seroconversion in sera of vaccinated chicken.

RESULTS

The data obtained in this investigation are illustrated in tables 1,2 and 3.

DISCUSSION

The oil adjuvant vaccine for controlling avian cholera disease, is of high protective value, efficient in stimulating the production of systemic humoral antibodies, Soliman (1983). By studying the most suitable storage conditions that maintains the efficacy and stability of improved polyvalent fowl cholera vaccine for poultry, it is evident that all the three batches that stored at 4 °C or 28-30 °C after 30, 60, or 90 days of storage, start to induce a systemic humoral antibodies after fifteen days postvaccination as shown in table (1). The geometric mean titres (GMT) of specific anti *Pasteurella multocida* antibodies increases till reach a maximum GMT on the 45th postvaccination day. There is a marked decrease of GMT in specific anti *Pasteurella multocida* antibodies, increases till reach a maximum GMT on the 45th postvaccination day. There is a marked decrease of GMT in specific anti *Pasteurella multocida* antibodies batches that stored at 28-30°C than those stored at 4 °C especially at 30th and 45th day postvaccination.

These results is further documented by potency testing in chicken and mice as acceptable protection, was noted in chicken vaccinated with avain cholera vaccine stored at 4 °C and 28-30°C (at room temperature) as shown in table (2).

It can be concluded from the previously mentioned results, that 4°C is the most suitable temperature for storage of avian cholera vaccine and the 28-30°C affect the vaccine by decreasing specific anti *Pasteurella multocida* antibodies. These findings are in complete agreement with those of Venkatesha et al (1989) who stated that when the haemorrhagic septicaemia vaccine was removed from storage and kept at ambient temperature, the vaccine should be used within one month. Observing the stability of the various lots of avian cholera vaccine revealed that, they were all stable and sterile when stored at 4°C or 28-30°C for 30, 60, and 90 days.

Regarding the duration of storage that maintains the efficacy, potency and stability of the various lots of improved polyvalent fowl cholera vaccine for poultry, it can be clearly seen from table (3) that the vaccine can induce

seroconversion and evoke a protection of 90% at the 6th month of stoarge. The protection still gave acceptable protection (65%) at the 15th month of storage. The results obtained in this study agreed with the previous finding of Nangia et al (1966) who recorded that *Pasteurella multocida* oil adjuvant vaccine was fully antigenic when stored at 7°C for 814 days. Also, Yadav and Ahooja (1986) stated that, after 6 months storage in the refrigerator, fowl cholera oil adjuvant vaccine conferred 80% protection and Vipulasiri et al (1982) also recorded that, loss of potency of *Pasteurella multocida* oil adjuvant vaccine at 4°C was slight after 6 months (3.4%) but greater after 12 months (63%).

Regarding the physical properties and sterility of examined batches, it was obvious that all batches remained stable and sterile up to 15 months after the date of manufacture.

It is evident from the results obtained in the present work that the improved polyvalent fowl cholera vaccine for poultry can be used safely and effectively up to 15 months post manufacture date when stored at 4°C.

REFERENCES

Azzzam, A.H.; Gergis, S.M.; and Kheir Eldin, A.M. (1992): Comparative efficacy and safety of polyvalent bacterins and avirulent live (CU) fowl cholera vaccines in protecting chickens and turkeys against fowl cholera. Proc. 2 Cong. Fac. Vet. Med., Cairo Univ. page 75-77.

Carter, G.R. and Rappy, D.E. (1962): Formalinized erythrocytes in the Haemagglutination test for typing *Pasteurella multocida.* Brit. Vet. J., 118, 289-292.

Fahmy, N. (1992): Studies on pasteurella infection in turkeys. M.D. Thesis (Poultry Dis.) Fac. Vet. Med., Cairo Univ.

Geneidy, A.A.; Lotfy, O. Nabil, E.; Abbassy, K. (1971): Control of fowl cholera with special reference to new oil adjuvant vaccine. Egypt. J. Vet. Med. Ass. 27, 121-126.

Gergis, S.M. (1978): Some immunizing properties concerning *Pasteurella multocida* in poultry. M.D. Thesis (Microbiology) Fac. Vet. Med., Cairo Univ.

Gergis, S.M.; Ghanem, I.M.; Nanaai, S.M.; Ali, N.M.; (1991): Evaluation of autogenous bacterin in controlling fowl cholera. Egypt. German. Conference.

Ghanem, I.A. (1986): Epidemiological studies on pasteurellosis in ducks. Ph.D. Thesis (Poultry and fish Dis.) Fac. Vet. Med. Zagazig Univ.

Gorgi, S.F. (1992): Comparison of efficency of different pasteurella vaccine used for production of ducks against fowl cholera in Egypt. Ph.D. Thesis (Poultry Dis.) Fac. Vet. Med., Assiut Univ.

Nangia, S.S.; Baxi, K.K.; Gulrajani. and Setherman, A.A. (1966): Haemorrhagic septicaemia oil adjuvant vaccine study. potency test in rabbits. Ind. Vet. J. 43, 279-287.

Ose, E.E. and Muenster, O.A. (1968): A method for evaluation of vaccines containing Pasteurella multocida. Am. J. Vet. Res. 29, 1863-1866.

Soliman, A.K. (1983): Immunological studies on the protective antigens of Pasteurella multocida of avian origin. Ph.D. Thesis (Microbiology) Fac. Vet. Med., Cairo Univ.

Venkatesha, M.D.; Giridhar, P.; Ananth, M. and Keshavamurthy, B.S. (1989): Effect of temperature and duration of storage on the keeping quality and potency of alum precipitated haemorrhagic septicaemia vaccine. Indian Vet. J. 66, 1097-1100.

Vipulasiri, A.A.; Wijewardana, T.G.; and Alwis, M.C.L.De. (1982): A note on the effect of storage temperature and time on the potency of haemorrhagic septicaemia oil adjuvant vaccine, Sri Lanka Vet. J. 30, 19-20.

Yadav, M.S. and Ahooja, M.L. (1986): Thermostability and keeping quality trials in rabbits with oil -adjuvant and multi-emulsion oil-adjuvant vaccine against haemorrhagic septicamia. Indian J. of Animal Sci. 56, 833-835.

Zaher, A.; El-Sabban, M.S.; Geneidy, M.; Sadek, I.M.; El-Agroudi, M.A., and Lotfy, O. (1974): The production of an oil adjuvant vaccine against pasteurella infection in turrkey. J. Egypt. Vet. Med. Ass. 34, 80-84.

Table (1) G.M.T. in serum of chicken vaccinated with avian cholera vaccine at different storage temperature for different periods.

Dayes post vaccination	Lot. No.	Stored at 4 0 C for			Stored at 28-30 0 C for		
		30 days	60 days	90 days	30 days	60 days	90 days
Fifteen days	A	184	160	184	160	160	160
	B	184	320	320	184	184	160
	C	160	184	320	160	160	184
Mean		176	221.33	274.6	168	168	168
Thirty days	A	453	453	453	422	422	320
	B	422	520	453	422	392	392
	C	520	520	640	392	392	392
Mean		465	497.6	515.33	412	402	368
Fourty five days	A	1114	1194	2079	668	686	422
	B	1194	2079	1114	640	640	453
	C	2079	1194	1114	640	640	520
Mean		1462.333	1489	1435.6	649.33	655.33	465

Table (2) Potency test for mice and previously vaccinated chickens using avian cholera vaccine stored for 30 , 60 and 90 days at 4° C and room temperature

Days of stotage	Lot No.	Vaccine stored at 4° C		Vaccine stored at 28-30° C	
		Protection % of chicken	Log protection in mice	Protection % of chicken	Log protection in mice
Thirty days	A	100	4.5	100	3.9
	B	100	4.5	90	3.9
	C	90	4.5	80	3.9
	Mean	96.6	4.5	90	3.9
Sixty days	A	100	4.2	90	3.4
	B	100	4.2	90	3.4
	C	90	4.2	80	3.4
	Mean	96.6	4.2	86.6	3.4
Ninety days	A	100	4.3	90	3.1
	B	100	4.3	80	3.1
	C	90	4.3	80	3.1
	Mean	96.6	4.3	83.33	3.1

N.B. Challenge was conducted 50 days after primary vaccination.

Table (3) The effect of duration of storage on the efficacy and potency of the various lots of improved polyvalent avian cholera vaccie for poultry.

Months of storage	Lot No.	3rd month	6th month	9th month	12 th month	15th month
Protection % of vaccinated chicken	A	100	90	90	80	70
	B	100	90	80	80	70
	C	90	90	70	70	60
Mean		96.6	90	80	76.6	66.6
GMT of passive haemagglutination at time of challenge	A	2079	2220	2220	1040	686
	B	1114	1114	1040	1040	640
	C	1114	1114	1114	1114	640
Mean		1435.6	1482.6	1458	1064.6	655.3
Protection % of the control non vaccinated chicken		0	0	0	0	0
Mean		0	0	0	0	0

N.B. Challenge was conducted 50 days after primary vaccination.