Beni-Suef Vet. Med. J. Vol. XI., No. (2) Oct., 2001 : (237 - 246).

PREPARATION OF A COMBINED VACCINE AGAINST CANINE DISTEMPER AND RABIES

By

Edries, S.M.; Guirguis, W. I.; Khodeir, M.H. and Attyat, M. Kotb

Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo

ABSTRACT

An inactivated cell culture combined vaccine was prepared against canine distemper and rabies. ERA strain of rabies virus propagated on BHK-21 and canine distemper virus propagated on Vero cells were inactivated by binary ethyleneimine (BEI) at 37°C. The inactivation time was 4 hours for rabies and 6 hours for canine distemper. The two viruses were inactivated simultaneously for 6 hours. As the protective dose of each vaccine was previously determined, the total virus protein was estimated in such doses and accordingly the two inactivated viruses were mixed together and alhydragel was added as adjuvant. Different groups of puppies (3-5 months of age) were vaccinated with single rabies inactivated vaccine, single canine distemper inactivated vaccine and the prepared combined rabies and canine distemper vaccine. Serological tests showed that all vaccinated animals exhibited good levels of specific antibodies against rabies and canine distemper without any antagonizing effect on their immune response in case of using combined vaccine. Also, the combined vaccine was found to be safe and immunogenic.

INTRODUCTION

Dogs are lovely pet animals and play a very important and serious role in our life. They often found in contact with our children and play a vital role in research and security as in police services. However, red light should be spotted on canine diseases where the major of them represent a public health hazard.

Rabies is the most dangerous canine disease. It is an acute fatal infectious disease caused by a filterable virus belonged to the family Rhabdovirus (**Hummeler *et al.*, 1968**). The disease is usually transmitted by biting of a rabied animal to a healthy one or man and mainly ends with death (**Smithcors, 1958 and Bear, 1975**).

Canine distemper is a major disease of dogs and other wild carnivora with the highest fatality rate beside rabies (**Appel and Montali, 1994**).

Rabies and canine distemper are endemic viral diseases in Egypt since long time (**Bucci *et al.*, 1982 and Bayoumi *et al.*, 1985**). The public risk of rabies was documented throughout the world and the domestic dogs were responsible for most human exposure and up to 98% of human fatalities were recorded due to rabies (**WHO, 1992**).

Suspicious that canine distemper might have a zoonotic importance as the human infection might be associated with multiple sclerosis (**Gaskell and Bennett, 1996**).

One of the main veterinary responsibilities is the control of such zoonotic diseases depending to a great distance on the use of specific potent vaccines. A great variety of pet animal vaccines were developed either in a live attenuated or inactivated forms. Such vaccines were found to be safe and immunogenic against rabies (**Wiktor, 1971; Larghi *et al.*, 1976 and Edries, 1994**) and against canine distemper (**Mansi, 1945 and Guirguis, 1991**).

It is well known that live attenuated vaccines induce good and long immunity against diseases. During the last few years, the use of inactivated instead of attenuated vaccines in animal vaccination, became compulsatory in many countries (**Pastoret and Falize, 1999**).

The use of combined or multivalent vaccines could simplify the prophylactics and control of diseases affecting pet animals and safe time, effort and cost (**Anon, 1989**). Examples of these vaccines were previously prepared by **Carmichael *et al.*, (1982); Churchill, (1987); Ackerman *et al.*, (1983); Khodeir *et al.*, (1998) and Edries *et al.*, (1999)** against rabies and other viral diseases like Rift Valley Fever and canine parvo. Also canine distemper with other viral vaccines were previously prepared by **Khodeir *et al.*, (1998) and Hamoda *et al.*, (2000)**.

The aim of the present study is to prepare an inactivated cell culture combined vaccine against both of rabies and canine distemper.

MATERIAL AND METHODS

1. Viruses:

1.1. Rabies virus strains:

1.1.1. Vaccinal strain:

ERA strain of rabies virus, propagated on BHK-21 cell culture was used for preparation of the inactivated vaccine (**Edries, 1994**). This cell culture strain was also used in serum neutralization test. It had a titre of 10^8 $TCID_{50}$/ml.

1.1.2. Challenge virus strain (CVS):

CVS was propagated in the brains of mice and had a titre of 10^7 $MICLD_{50}$/0.03ml (mouse intracerebral lethal dose). It was used at a final concentration of 100 $MICLD_{50}$ in the test of National Institute of Health (NIA) to detect the potency of rabies vaccine.

1.2. Canine distemper strains:

1.2.1. Vaccinal strain:

A living cell culture adapted canine distemper virus propagated on Vero cells was used for the inactivated vaccine and in serum neutralization test. It was adapted to cell culture by **Guirguis, (1991)**. It had a titre of 10^7 $TCID_{50}$/ml.

1.2.2. Virulent strain:

Synder-Hill strain of canine distemper virus was kindly supplied by James-A-Baker, Institute for Animal Health, USA. It was used as a virulent strain in the challenge test of vaccinated animals. It had a titre of 10^5 EID_{50}/ml (Egg Infective Dose).

2. Virus inactivation and inactivated vaccines preparation:

Batches of cell culture rabies (ERA-strain) and canine distemper viruses were subjected to a process of inactivation using binary ethyleneimine at a final concentration of 0.01 M according to **Edries, (1994)**. The inactivation process was stopped using 20% sodium thiosulphate at a final concentration of 2%. The inactivation time was found to be 4 hours for rabies and 6 hours for canine distemper virus. Alhydragel was added as an adjuvant at a concentration of 40% to single and combined vaccines according to **Edries, (1994)**.

The combined vaccine was prepared by mixing the two inactivated viruses in a manner ensuring that the final volume contains the protective doses depending on the amount of viral protein in each vaccinal dose which previously determined by **Edries, (1994) and Guirguis, (1991)**.

3. Determination of the viral antigen protein:

The protein content was determined in both of cell culture media, uninfected cell suspension in saline and infected fluids, according to **Weichselbaum, (1946)**. The media protein plus cell protein were subtracted from the infected fluid protein to obtain the viral antigen protein.

4. Quality control tests of the prepared vaccines:

The prepared vaccine batches were subjected to quality control tests including the freedom of foreign contaminants, safety and potency according to the direction of **FAO (1994)**.

NIH test was done according to **Larghi and Nebel, (1980)** to detect the potency and safety of rabies vaccines in mice. The antigenic value of NIH should not be less than 0.3. Also, the safety and potency of such vaccines were done in dogs for canine distemper.

5. Animals:

5.1. Mice:

200 weaned albino Swiss mice were used to test the safety of rabies vaccines according to **British Pharmacopoeia (1985)** and the potency using NIH test according to **Larghi and Nebel, (1980)**.

5.2. Dogs:

25 puppies (3-5 months of age) were used in the present study divided into different groups as follow:

Group (1): Consisted of 9 dogs where the double dose of each vaccine was inoculated in each of 3 dogs to test the safety of such vaccine.

Group (2): Consisted of 3 dogs was vaccinated with the single inactivated canine distemper vaccine (1ml S/C).

Group (3): Containing 3 dogs was inoculated with the single inactivated rabies vaccine (1ml S/C).

Group (4): Consisted of 6 dogs was vaccinated with the combined inactivated canine distemper and rabies vaccine (2ml S/C).

Group (5): Containing 4 dogs was kept unvaccinated as test control.

All animals were kept under hygienic measure and daily clinical observation for 8 weeks post vaccination.

Blood samples were obtained from all animals per week intervals where the separated sera were serologically tested for the induced antibodies.

Group (2) and 2 dogs of group (4) were challenged with the virulent canine distemper virus at the dose of 10^5 EID_{50}/dog, 21 days post vaccination.

6. Serum neutralization test (SNT):

Micro-neutralization test was carried out according to **Bass *et al.*, (1982)** to demonstrate and estimate the induced antibodies in vaccineated animals. BHK-21 cell culture was used for rabies SNT and Vero cell culture was used for canine distemper SNT.

RESULTS AND DISCUSSION

The golden goal of all medical researchers could be termed as "control and eradication of infectious zoonotic diseases". Rabies and canine distemper are two major viral infectious diseases causing a great public health hazard (**WHO 1992 and Gaskell and Bennett, 1996**).

Veterinary vaccinology is an interesting rapidly developed research field not only to control animal diseases, but also to solve public health problems. So, in the present study, the researchers tried to prepare an inactivated cell culture combined vaccine against rabies and canine distemper.

In the present study binary ethyleneiinine (BEI) was used as viral inactivator, where it suppress the infectivity of viruses leaving their antigenic structure and viral protein unaffected (**Sashi and Mohanty, 1981**).

Table (1) showed that the viral antigen proteins in rabies; canine distemper and combined vaccinal protective doses were 12.5, 21.1 and 25 mg/ml. These findings come in complete agreement with those of **Bradford, (1976); Killington *et al.*, (1996) and Edries *et al.*, (1999)**.

The experimental results revealed that a complete viral inactivation of rabies virus and canine distemper virus was 4 and 6 hours, respectively using BEI at 37°C (Table 2). Similar results were recorded for rabies inactivation by BEI **(Edries, 1994 and Edries *et al.*, 1999)**. There were unavailable data discussed canine distemper inactivation by BEI.

All prepared vaccines were found to be free from foreign contaminants (aerobic and anaerobic bacteria, fungi and mycoplasma). The results of NIH (Table 3) revealed that the antigenic value of rabies virus in prepared vaccine was 2.1 and 2.3 for single and combined vaccines, respectively. These values are higher than those recommended by **Larghi and Nebel, (1980)** who suggested that the antigenic value of inactivated canine distemper vaccines should not be less than 0.3. Also, Table (4) showed that the prepared vaccines are safe for vaccinated dogs where they did not show any abnormal clinical signs. In addition, canine distemper was found to be a potent vaccine either in a single or combined form with rabies where protection ratios of 100% were recorded (Table 5).

So, the previous discussed results showed that the prepared inactivated rabies and canine distemper vaccine either in the single or combined forms, were found to be safe and immunogenic. These findings agree with the recommended conditions of **WHO (1992) and FAO (1994)** and confirmed by **Edries, (1994) and Edries *et al.*, (1999)**.

The seroconversion using SNT (Table 6) revealed that there was no adversable effect of rabies or canine distemper on the immune response of vaccineated dogs to the other virus vaccine. All vaccinated animals exhibited good levels of specific neutralizing antibodies from the 1st week post vaccination recording peak titres by the 4th - 5th week and remain constant allover the experimental period (8 weeks). Such results were found to be similar and confirmed by those obtained previously by **Edries, (1994); Edries *et al.*, (1998); Khodeir *et al.*, (1998); Khodeir (1999) and Edries *et al.*, (1999)** indicating that combination of rabies or canine distemper with other viral vaccines did not affect or interfere with the immune response of vaccineated animals to other antigens.

So, it could be concluded that the prepared inactivated combined rabies and canine distemper vaccine is a safe and potent vaccine protect dogs against the two diseases safely with good levels of immunity.

REFERENCES

Ackerman, O.; Stegman, H. and Jaeger, O. (1983): Simultaneous immunization of dogs against parvo virus, distemper, rabies, contagious hepatitis and leptospirosis. Blauen Hefte Fur Den Tierazt., 67: 302-308.

Anon, A. (1989): Canine and feline immunization guide lines. J. Amer. Vet. Med., 195 (3): 314-317.

Appel, M.J. and Montalim R.J. (1994): Canine distemper and emerging disease in exotic species. Amer. Ass. Zoo. Vet., October 1994: 22-27.

Bass, E.P.; Gill, M.A. and Beckenhauer, W.H. (1982): Development of a modified live canine origin parvo virus. J. Amer. Vet. Med. Ass., 181 (9): 909-913.

Bayoumi, A.H.; Ahmed, L.S.; Ibrahim, M.K.H. and Mahmoud, A.Z. (1985): Studies on canine distemper among stray dogs in Assiut governorate. Assiut Vet. Med. J., 15 (29): 113-119.

Bear, G.M. (1975): The natural history of rabies. Acad. Press New York. 3rd Ed. pp. 290.

Bradford, M.M. (1976): A rapid and sensitive method for the quantitation of micrograms quantities of protein utilizing the principle or protein-dye binding. Anal. Biochem., 72: 248-252.

British Veterinary Pharmacopoeia (1985): "Rabies vaccine". P.181.

Bucci, T.J.; Botros, A.M. and El-Molla, M. (1982): Canine parvo infection: A brief review and report or first case in Egypt. J. Egypt. Vet. Med. Assoc., 42 (1): 21-25.

Carmichael, L.E.; Joubert, J.C. and Pollock, R.V. (1982): A modified live canine parvovirus: immune response. Corn. Vet., 73: 13-29.

Churchill, A.E. (1987): Preliminary development of a live attenuated canine parvo virus vaccine from an isolate of British origin. Vet. Rec., 120: 334-339.

Edries, S.M. (1994): Studies on preparation of inactivated tissue culture anti-rabies vaccine. Ph.D. Thesis, Microbiology, Fac. Vet. Med., Cairo Univ.

Edries, S.M.; Attyat, M. Kotb and Habashi, Y.Z. (1998): Studies on the immune response post vaccination with inactivated tissue culture rabies vaccine. Vet. Med. J., Giza, 46 (4B): 743-749.

Edries, S.M.; Attyat M. Kotb; Habashi, Y.Z. and El-nakashly, S. (1999): Some studies on preparation of a combined vaccine against rabies and parvo viral diseases. Alex. J. Vet. Sci., 15 (3): 591-599.

FAO (1994): Quality control of rinderpest cell culture vaccine. Standard Operating Procedures. FAO Report, No. 118.

Gaskell, R.M. and Bennett, M. (1996): Feline and canine infectious diseases. Blackwell Sci. Inc., USA, 111-117.

Guirguis, W.I. (1991): Trials for preparation of a vaccine against canine distemper. Ph.D. Thesis, Microbiology, Fac. Vet. Med., Cairo Univ.

Hamoda, F.K.; El-Gallad, S.B.; Attyat, M. Kotb; Edries, S.M.; Amani, A. Saleh and Guirguis, W.I. (2000): Studies on simultaneous vaccination of dogs with some pet animal vaccines. Vet. Med. J. Giza, 48 (4): 647-657.

Hummeler, K.; Tomassini, N.; Sokol, K.; Kuwert, F. and Koprowski, H. (1968): Morphology of nucleoprotein component of rabies virus. J. Virol., 2: 1191-1194.

Khodeir, M.H. (1999): Studies on vaccination of farm animals (cattle, horse, sheep) in addition to dogs and cats with inactivated tissue culture rabies vaccine. Beni-Suef Vet. Med. J., 9 (3-A): 111-120.

Khodeir, M.H.; Attyat M. Kotb; Guirguis, W.I. and habashi, Y.Z. (1998): Preparation of a bivalent vaccine against canine distemper and canine parvo viruses. 4th Vet. Med. Zag. Cong., 152-160.

Khodeir, M.H.; Khirat A. Elian; Edries, S.M. and Gehan, K.M. (1998): Preparation of a combined vaccine against rabies and Rift Valley Fever. 4th Vet Med. Zag. Cong., 209-216.

Killington, R.A.; Stokes, A. and Hierhdzer, J.C. (1996): Virology methods manual, Chapter 4, virus Purification, Acad Press, 71-89.

Larghi, O.P. and Nebel, A.E. (1980): Rabies virus inactivation by binary ethyleneimine: New method for inactivated vaccine production. J. Clin. Microbiol. Jan, P. 26-33.

Larghi, O.P.; Savy, V.L.; Nebel, A.E. and Rodriguez, A. (1976): Ethyleneimine inactivated rabies vaccine of tissue culture origin. J. Clin. Microbiol., 1: 26-33.

Mansi, W. (1945): Bacteriology of distemper. M.D. Thesis, Food Univ. Cairo, Egypt.

Pastoret, P.P. and Falize, F. (1999): Viral Veterinary Vaccines. Dev. Biol. Stand., 101: 73-78.

Sashi, B.M. and Mohanty, K.D. (1981): Vet. Virology, Chapter 7, P. 8.

Smithcors, J.F. (1958): Purification of rabies virus grown in tissue culture. J. Virol., 2: 836-849.

Weichselbaum, T.E. (1946): An accurate and rapid method the determination of protein in small amounts of blood serum and plasma. Am. J. Clin. path., 16: 40-49.

WHO (1992): World Survey of rabies. No. 25 WHO Rabies 29-203: Geneva.

Wiktor, T.J. (1971): Nature and properties of rabies virus. P. 37-51 in Y, Nagan and F.M. Daveport (ed). Rabies. Univ. Park Press, Baltimore.

Table (1): Antigenic viral proteins in prepared vaccines

Vaccine type	Antigenic viral protein (mg/ml)
Single rabies Vaccine	12.5
Single canine distemper vaccine	12.1
Combined rabies and canine distemper vaccine	25.0

Table (2): Inactivation of rabies and canine distemper virus using BEI at 37°C

Time of inactivation *	Virus titer log_{10} $TCID_{50}$/ml	
	Rabies virus	Canine distemper virus
0	7	6
1	5	5
2	3	4
3	1	3
4	0	2
5	0	1
6	0	0

HPI = Hours Post Inactivation.

Table (3): Potency of inactivated rabies virus in prepared vaccines using NIH test in mice.

Vaccine type	NIH Antigenic value
Single inactivated rabies vaccine	2.1
Combined rabies and canine distemper inactivated vaccine	2.3

Table (4): Safety of prepared vaccines in dogs

Prepared vaccine	Number of vaccinated dogs	Number of animals showing clinical abnormalities
Single rabies Vaccine	3	0
Single canine distemper vaecine	3	0
Combined rabies and canine distemper vaccine	3	0

Table (5): Potency of canine distemper in the prepared vaccine

Used vaccine	Number of vaccinated dogs	No. of challenged dogs	Survived dogs	Protection %
Single canine distemper vaccine	3	3	3	100
Combined rabies and canine distemper vaccine	6	3	3	100
Unvaccinated control	2	2	0	0

Table (6): Serum neutralizing antibody titers in vaccinated dog groups.

Used vaccine	Mean neutralizing antibody titer*/weeks post vaccination																	
	0 WPV		1 WPV		2 WPV		3 WPV		4 WPV		5 WPV		6 WPV		7 WPV		8 WPV	
	R	CD	R	CD	R	CD	R	CD	R	CD	R	CD	R	CD	R	CD	R	CD
Single rabies vaccine	0	0	4	0	16	0	64	0	128	0	256	0	256	0	256	0	256	0
Single canine distemper vaccine	0	0	0	2	0	4	0	8	0	16	0	32	0	32	0	32	0	32
Combined rabies and canine distemper vaccine	0	0	2	2	16	8	32	16	128	32	128	32	256	32	256	32	256	32
Unvaccinated control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Antibody titre = The reciprocal of serum dilution which neutralize and inhibited the CPE of 100-200 $TCID_{50}$ of the virus.
WPV = Week Post Vaccination. R = Rabies. CD = Canine Distemper

الملخص العربى

تحضير لقاح مركب ضد كل من السعار وحصبة الكلاب

سمير محمد إدريس وصفى إبراهيم جرجس
محمد حسن خضير عطيات محمد قطب
معهد بحوث الأمصال واللقاحات البيطرية – العباسية – القاهرة

خلال هذه الدراسة تم تحضير لقاح مركب نسيجى مثبط ضد كل من مرض السعار (داء الكلب) وحصبة الكلاب (الديستمبر) حيث تم تثبيط فيروس السعار المنمى على خلايا كلى الجربوع وفيروس حصبة الكلاب المنمى على خلايا كلى القرد الأخضر الأفريقى حيث وجد أن مدة التثبيط اللازمة هى ٤ ساعات للأول و ٦ ساعات للثانى باستخدام مادة البينارى إيثيلين آمين فى درجة ٣٧°م ثم تم تثبيطهما معاً لمدة ٣ ساعات. وبمعرفة الجرعة الواقية لكل لقاح على حدة من الدراسات السابقة تم تحديد نسبة البروتين الكلى اللازمة من كل فيروس فى اللقاح المثبط. وعلى ذلك تم خلط الفيروسين المثبطين معاً وتخفيفهما بحيث تحتوى جرعة التحصين على كمية البروتين الفيروسى اللازمة كجرعة واقية. وإضافة مادة الهيدراجيل كمساعد للقاح. وتم تحصين مجموعات من الكلاب (٥-٨ شهور) بلقاح الديستمبر المثبط ولقاح السعار المثبط (كلقاحات فردية) وباللقاح المركب مع ترك ضوابط للتجربة بدون تحصين. أظهرت نتائج التجارب السيرولوجية أن كل الحيوانات المحصنة تكتسب مستويات جيدة من المناعة ضد كلا المرضين دون تداخل بين الفيروسين ودون تأثير عكسى على الاستجابة المناعية للحيوانات. كما أن اللقاح الثنائى آمن وفعال. وعلى هذا يمكن القول بأن اللقاح المركب ضد كل من السعار والديستمبر لقاح يصلح لتحصين الكلاب ضد كلا المرضين بأمان وكفاءة مناعية جيدة.