PREPARATION OF AN INACTIVATED VACCINE AGAINST HARD PAD DISEASE (CANINE DISTEMPER) IN CATS FROM THE LOCAL ISOLATE

GUIRGUIS,W.I.;AZAB,A.M.H;KHODEIR,M.H. AND DAOUD,A.M.

VET.SER.VAC.RES.INST.,ABBASSIA,CAIRO

ABSTRACT

The local isolate of canine distemper (Hard pad) virus isolated from a stray cat and designed as CD-Abbassia/2001, was propagated in vero cell culture and inactivated with Binary Ethylene Amine (BEA) where the Alhydragel was added as adjuvant. Different doses (1,2&3ml) of such vaccine were inoculated S/C in different groups of cats (local breed) first in the form of single dose and second time in the form of 2 doses with apart 2 weeks in between. Three weeks later, the challenge test revealed that the most suitable protective dose is 2ml-inoculated S/C followed by a booster dose after 2 weeks. Vaccination of cats with 2 doses resulted in a protection ratio of 100% while the use of one dose induced a 60% protection. Higher dose (3ml) showed the same protection ratio as 2ml while the lower dose (1ml) did not induce a permissible protection. Serum neutralization test indicated that vaccinated cats exhibited good levels of specific neutralizing antibodies reached up to a titer of 32.Also the quality control tests revealed that the prepared vaccine was free from foreign contaminants, safe and immunogenic.

INTRODUCTION

Canine distemper is a contagious disease of a high fatality affecting puppies and caused by a Morbillivirus of the family Paramyxoviridae (Sashi, et.al.1981). The disease can affect animals other than the family Canidae (El-Hammamy, et.al.1997). Also, it was found to be of wide spread in large cats (Myers, et.al.1997).

Canine distemper in cats was described as Hard pad disease for the first time in Egypt (Guirguis,et.al.2001) in a stray cat which showed the symptoms of depression, emaciation, slight nasal and ocular discharges, skin vesicles on the preneal region and keratinization of the foot pad of the four limbs. The causative agent was isolated and identified as canine distemper virus in cats and named CD-Abbassia/2001. These findings spotted the light on the role of cats in the transmission of canine distemper. So, it was taken in consideration the essentiality to prepare a specific vaccine to control the disease in cats and prevent its transmission to dog populations where active immunization is considered the corner stone in controlling of infectious diseases.

Among canine distemper vaccines; many forms were developed successfully and have a good immunogenicity. Such vaccines were prepared in different forms including single live attenuated (Mansi,1945; Haig,1956 and Guirguis,1991); in a bivalent form with other antigens as canine Parvo (Khodeir,et.al.1998), with rabies (Edries,et.al.,2001); and inactivated virus vaccine (Laidlaw and Dunkin,1928; Sir Christopher,1964; Naglaa,2001and Amani,et.al 2002).

The main goal of the present study is to prepare an inactivated vaccine against canine distemper (Hard pad disease) in cats.

MATERIAL AND METHODS

1-Virus:

CD-Abbassia/2001 virus strain (Guirguis,et.al.2001) was used as a local strain of canine distemper isolated from cat. It was passaged 3 times in VERO cells and had a titer of 10^6 $TCID_{50}$/ml. The original virus was used as a virulent strain for the challenge of vaccinated cats while the cell culture passaged one was used for vaccine preparation.

2-Virus titration:

It was applied using the microtiter technique according to Rossiter, et.al. (1985) and the virus titer was calculated according to Reed and Menuch (1938).

3-Virus inactivation:

1M Binary Ethylene Amine (BEA) at the ratio of 3% and sodium thiosulphate (20% solution) was used at the ratio of 2% as a stopping solution according to Edries,et.al.(2001). The time of complete virus inactivation was estimated depending on virus titration on different time intervals (from 1 to 10 hours).

4-Preparation of an inactivated vaccine:

Different ratios of Alhydragel (2% aluminum hydroxide gel) as 10,20 and 30 were added to portions of the infected fluid before the inactivation process to detect the most suitable ratio for complete virus adsorption. Such ratio was found to be 20%. So, the prepared vaccine was formed of 80% inactivated virus fluid and 20% alhydragel as an adjuvant.

5-Quality control tests:

Sterility (freedom of bacteria, fungi and mycoplasma); safty (by inoculation of the double dose '4ml') and potency (by the challenge test) were carried out according to FAO (1994).

6-Animals:

6.1-Cats:

22 local breed cats of about 4 months old, were used in the present study. All of them were screened for canine distemper antibodies and found to be free from them. The cats were divided into 4 groups as follow:

a-Group-1 including 9 cats subdivided into 3 subgroups (3cats in each) where different doses (1,2&3ml) of the prepared vaccine were inoculated s/c one time in a cat subgroup.

b-Group-2 of 9 cats was also subdivided into 3 subgroups (3cats in each) inoculated with similar doses for 2 times with 2 weeks in between.

c-Group-3 of 2 cats was used for safety test where each cat received the double protective dose of the prepared vaccine.

d-Group-4 of 2 cats was kept unvaccinated as test control.

All cats were kept under hygienic measures in isolated cages and received balanced diet. Daily clinical examination was performed including all animals.

6.2-Mice:

25 weaned Swiss mice were used to test the safety of the prepared vaccine. 0.5ml of such vaccine were inoculated intrapretonially in each of 20 mice while 5 mice were kept as control.

7-Samples:

Serum samples were obtained from all animal groups before vaccination and weekly after vaccination for 4 times then monthly for 4 months post the last vaccination. The induced antibodies were estimated in such sera using the microneutralization test according to Rossiter,et.al.(1985) and the antibody titers were calculated as the reciprocal of serum dilution which neutralized and inhibited the CPE of 100-200 $TCID_{50}$ of the virus according to Singh,et.al. (1967).

8-Challenge test:

All vaccinated and unvaccinated control cats were challenged using the virulent strain inoculated intranasally 3 weeks post the last dose of vaccination. The challenge dose was $10^3 TCID_{50}$/cat according to Guirguis (1991).

RESULTS AND DISCUSSION

The inactivation process of CD-Abbassia/2001 (the local isolate of canine distemper virus causing hard pad disease in cats) using 3% of 1M Binary ethylene Amine (BEA) revealed that a complete inactivation of the virus was obtained 6 hours post the beginning of the inactivation process (Table-1).Edries,et.al.(2001) and Amani,et.al.(2002) reported that such time was 7 hours using the same molarity and the same ratio of BEA . This difference could be attributed to the virus strain and virus titer used.

It was found that 20% Alhydragel as adjuvant resulted in complete virus adsorption (Table-2) coming in complete agreement with Edries,et.al.(2001); Naglaa (2001) and Amani,et.al.(2002).

The prepared vaccine was found to be free from foreign contaminants (bacteria; fungi and mycoplasma).Inoculation of the double dose did not affect the health condition of inoculated cats indicating the safety of the vaccine. In addition; after the detection of the protective dose; it was found that vaccinated cats withstood the challenge with the virulent virus while unvaccinated cats showed sings of illness and death. These findings followed up and agree with the directions and conditions reported for vaccine preparation by the FAO (1994).

The experimental results showed that the most suitable protective dose of the prepared vaccine is 2ml inoculated s/c and followed by a booster one after 2 weeks resulted in 100% protection (Table-3); where 1ml and 2ml or even 3ml given one time resulted in death of challenged cats and lower protection (60%). In fact, there were no available literatures that discuss preparation of an inactivated vaccine against hard pad disease or vaccination of cats against it; but the same dose was recorded for dogs from inactivated canine distemper vaccines by Edries,et.al.(2001); Naglaa (2001) and Amani,et.al.(2002).

Serum neutralization test (SNT) revealed that the vaccinated cats (with 2 doses) exhibited specific neutralizing antibodies began to appear by the 2^{nd} week post vaccination (4-8) and reach their peak by the 4^{th}-5^{th} week post the second dose (Table-4). Similar results were obtained in dogs vaccinated by inactivated canine distemper vaccine by Edries,et.al. (2001) and Amani,et.al. (2002) but higher levels were recorded by Guirguis (1991); Guirguis,et.al.(1999) and Khodeir,et.al. (1998) when they used live attenuated vaccines. The obtained values of antibodies in the present study could be considered of protective levels where they overcame the challenge with the virulent virus while unvaccinated animals showed signs of illness or death.

So, it could be concluded that the prepared inactivated vaccine against hard pad disease is a safe and immunogenic vaccine protects cats against the disease and help to prevent its transmission to dogs.

Table (1):Inactivation of CD-Abbassia/2002 virus strain

Hours of inactivation	The original virus titer ($TCID_{50}$/ml)	The virus titer post the inactivation	Log 10 difference
0	6	6	0
1	6	5	1
2	6	4	2
3	6	3	3
4	6	2	4
5	6	1	5
6	6	0	6

Table (2): Virus adsorption to Alhydragel

Alhydragel %	Residual virus titer
10	$10^2 TCID_{50}$/ml
20	$<10 TCID_{50}$/ml
30	$<10 TCID_{50}$/ml

Table (3): Potency and detection of the protective dose of the prepared inactivated Hard pad vaccine for cats

Cat groups	Vaccination dose	Number of vaccinations	Protection% post challenge
a-Subgroup-1	1ml	1	0
a-Subgroup-2	2ml	1	0
a-Subgroup-3	3ml	1	0
b-Subgroup-1	1ml	2	60
b-Subgroup-2	2ml	2	100
b-Subgroup-3	3ml	2	100
c-Unvaccinated	0	0	0

Table (4): Mean neutralizing antibody titers in the sera of vaccinated cats with the protective dose

Cat group	Mean serum neutralizing antibody titer*/WPV**									
	1wpv	2wpv	3wpv	4wpv	5wpv	6wpv	7wpv	11wpv	12wpv	16wpv
a-Subgroup-2	0	0	4	4	8	8	8	8	4	4
b-Subgroup-2	0	4	Boostering	8	16	32	32	32	32	32
c.Unvaccinated	0	0	0	0	0	0	0	0	0	0

*Antibody titer= the reciprocal of serum dilution which neutralized and inhibited the CPE of 100-200$TCID_{50}$ of the virus.

**WPV= weeks post vaccination.

REFRENCES

Amani, A.Saleh; Azab,A.M.; El-Gallad,S.B. and Khodeir,M.H.(2002):
Preparation of a trivalent dog vaccine (Rabies, Canine distemper and Canine Parvo).
J.Egypt.Vet.Med.Assos. (Under press).

Edries,S.M.; Guirguis,W.I.; Khodeir,M.H. and Attyat,M.Kotb (2001):
Preparation of a combined vaccine against canine distemper and rabies.
Beni-Suef Vet.Med.J.Vol.XI.,No.(2): 237-246.

El-Hammamy,M.M.; Kamel,A.M.; and Guirguis,W.I. (1997):
Clinical and pathological studies of experimentally induced canine distemper in free ranging foxes (Vulpes vulpes).
2nd Sin.Conf.Vet.Med.Alexan.1997 (5): 581-591.

FAO (1994):
Quality control of rinderpest cell culture vaccine.
Standard operating procedures. FAO report No.118.

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

Guirguis,W.I.; Azab,A.M.; Khodeir,M.H. and Daoud,A.M.(2001):
Isolation of the causative agent of hard pad disease in cat (canine distemper): A preliminary study to prepare a specific vaccine.
2nd Sint.Conf.Fac.Vet.Med.Beni-Suef, 465-477.

Haig,D.A. (1956):
Canine immunization with avianized virus.
Onderstepoort J.Vet.Res.27.

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 Sint.Conf.Fac.Vet.Med.Zagazig,26-28 August 1998, Hurghada,152-160.

Laidlaw,P.P. and Dunkin,G.W. (1928):
Studies on dog distemper.III-The nature of the disease.
J.Comp.Path.Ther.Vol.41,pp.222-230.

Mansi,W.(1945):
Bacteriology of distemper.
M.Vet.Thesis, Foad Univ.

Myers,D.L.; Zurbrigsen,A.; Lutz,H. and Posichil,A. (1997):
Distemper not a new disease in lions and tigers.
Clin.Diag.Lab.Immun.(1997) Mar.4;(2): 180-184.

Naglaa,I.A.(2001):
Studies of the preparation of an inactivated canine distemper vaccine.
Ph.D.Thesis, (Virology),Fac.Vet.Med.,Cairo Univ.

Reed,L.J. and Menuch,H. (1938):
A simple method of estimating fifty percent end points.
Am.J.Hygien,27:493.

Rossiter,P.B.; Jesset,D.M. and Taylor,W.P. (1985):
Microneutralization system for use with different strains of pest des petites ruminants virus and rinderpest virus.
Trop.Anim.Hlth.Prod.,17 (2): 75-81.

Sashi,B.; Mohanty,M. and Suskantak,D.(1981):
Distemper.
Vet.Microbiol.pp. 215-217.

Singh,K.V.; Osman,O.A.; Ivon,F.E. and Thanaa,I.Baz (1967):
Colostral transfer of rinderpest neutralizing antibodies to offspring of vaccinated dams.
Can.J.Comp.Med.Vet.Sci.,32;295-298.

Sir Christopher,A.(1964):
Viruses of vertebrates.London.

تحضير لقاح منشط ضد مرض جفاف الكف (الديستمبر) فى القطط من العترة المعزولة محليا لفيروس ديستمبر الكلاب

د/وصفى إبراهيم جرجس-أ.د/ عادل محمد حسن عزب-أ.د/ محمد حسن خضير
أ.د/ احمد محمود داود

معهد بحوث الأمصال واللقاحات البيطرية
العباسية- القاهرة

تم اكتشاف مرض جفاف الكف (الديستمبر) فى القطط لأول مرة فى مصر عام ٢٠٠١فى قطة ضالة حيث تم عزل الفيروس المسبب وتصنيفه كعترة محلية من فيروس ديستمبر الكلاب فى القطط سميت عترة عباسية/٢٠٠١.هذا وقد تم تنمية وأكثار الفيروس فى خلايا كلى القرد الأخضر الأفريقى ثم تثبيطه بمادة البينارى إيثيلين أمين مع أضافة الهيدراجيل كمساعد .وبحقن جرعات مختلفة(١و٢و٣سم^{٣})من هذا اللقاح تحت الجلد فى مجموعات مختلفة من القطط تارة مرة واحدة وأخرى مرتين (أسبوعين بين الجرعتين) ثم أجراء أختبار التحدى (ثلاثة أسابيع بعد أخر جرعة) تبين ان أنسب جرعة هى ٢سم^{٣} تعطى مرتين بينهما فترة أسبوعين حيث اعطت هذه الطريقة من التحصين نسبة حماية ١٠٠% بينما كانت نسبة الحماية عند أعطاء جرعة واحدة ٦٠% ولم تعطى الجرعات الأقل أية حماية . كما أظهرت أختبارات الجودة أن اللقاح المحضر خالى من الملوثات وأمن حيث أنه لم يتسبب فى ظهور أعراض مرضية على القطط قيد التجربة . كما ثبتت فعالية اللقاح باختبار المصل المتعادل الذى اوضح تكون أجسام مناعية نوعية فى القطط المحصنة تصل الى معيار ٣٢ وتتغلب على التحدى بالفيرس. وعلى ذلك يمكن القول بأن هذا اللقاح يصلح لحماية القطط ضد الديستمبر ويساعد فى عدم انتشاره للكلاب .