

ORAL VACCINATION OF PUPPIES WITH THE CORE VACCINES (CANINE DISTEMPER; CANINE PARVO; CANINE HEPATITIS AND RABIES VACCINES)

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ABSTRACT

Four cell culture vaccines were prepared as single vaccines against canine distemper (CD); Canine parvo (CP); canine hepatitis (CH) and rabies. The first three vaccines were prepared as attenuated vaccines on Vero cells while rabies vaccine was prepared in BHK-21 cells as attenuated and inactivated vaccine. Such vaccines were used to vaccinate puppies' parentally through the subcutaneous route and another time in the form of fish cake baits. In addition tetra dog vaccine was prepared from these vaccines and tested in puppies through the subcutaneous and oral routes. All of the prepared vaccine forms were found to be safe inducing no abnormal clinical signs post vaccination. Vaccinated puppies exhibiting good levels of specific CD; CP; CH and rabies antibodies, as measured by serum neutralization test. Such antibodies were capable to protect vaccinated puppies against challenge virulent with virus strains indicating that the oral delivery bait vaccines can protect dogs against the common contagious viral diseases and help to keep these infectious diseases under control.

INTRODUCTION

Vaccination has proved itself over the 120 years to be the most efficient and cost-effective strategy for public health improvement by controlling the infectious disease (*Tizard, 2000 and Stephenson, 2001*)

The American Animal Hospital Association (AAHA) Canine vaccine guidelines 2006 classified canine vaccines as core (Universally recommended) and non-core (not recommended). Components considered core vaccines for dogs are canine distemper canine adenovirus, Canine parvovirus type - 2 and Rabies vaccines globally. Rabies continuous to be an important disease of human and animals. It is one of the dead list human diseases. It is an acute fatal viral encephalomyelitis disease, caused by a filterable virus that belongs to family Rhabdoviridae (*Hummeler, et al. 1968*).

Canine distemper (CD) was placed beside Rabies as diseases causing the highest fatality among dogs and other wild carnivores (*Appel and Montali, 1994*). The disease characterized by respiratory diseases, fever and death (*Smith and Lauffer, 1962*) and in adult human making a chronic inflammatory bone disorder "Paget's disease (*Cartwright et al., 1993 and Reddy, et al. 1996*). It is caused by a virus belong to family Paramyxoviridae (*Sashi and Dulta 1981*).

Canine parvo(CP)is a highly contagious,viral disease characterized by bloody diarrhea vomiting and emaciation that usually ends with death especially in young puppies (*Johnny, 1998*). The disease is caused by a virus belong to family Parvoviridae (*Appel and Gillespie, 1972*).

Infectious canine hepatitis (ICH) or Rubath's disease is a serious disease characterized by respiratory, ocular disease encephalopathy, chronic hepatitis and interstitial nephritis. The disease is caused by canine adenovirus type-1 (CAV-1) virus belong Adenoviridae family (*Greene, 1998*).

However, most currently available vaccines are delivered by injection, which makes mass immunization more costly and less safe, particularly in resources-poor developing countries (*Wang and Coppel, 2008*). Oral vaccines have several attractive features compared with parenteral vaccines including easy and painless administration; more important only mucosal vaccines consistently promote immune responses at the most common sites of entry of infectious agents (*Prosper, et al 2005*).

Since the 1960's, oral rabies vaccination was developed as a tool to control Rabies. The first field trial was a blood in Switzerland to control rabies in foxes (*Steek, et al. 1982*). Muller mentioned that the oral Rabies vaccine success in eradication of Sylvatic rabies in Western Europe (*Muller, 2000*).

Also, combining antigens from a variety of pathogens to form effective "multi-component vaccines" is one of the challenges of modern vaccine research. There is a potential problem for multi-component vaccines in limitations on the volume of materials that can be injected. Oral administration may circumvent this problem, as well as avoid many of the adverse effects observed when vaccines are administered parentally (*Noemi, et al. 1995*).

So, attention has turned to the application of this technology to domestic dog populations particularly those characterized by a high proportion of dog in accessible to traditional mass parenteral vaccination methods (*WHO, 1998*).

The main goal of this study is to prepare and evaluate the efficacy of tetra dog vaccine "Rabies, CDV, CPV and CAV-1) administrated orally to puppies.

MATERIAL AND METHODS

1- Viruses:

1.1 Vaccinal strains:

Cell culture adapted rabies virus strain ERA propagated on BHK₂₁ cell line (*Abdelseth, 1964*); Rock born canine distemper virus strain (CDV) (*Rockborn, 1958*); Canine parvo virus (CPV₃₉) (*Parrish, et al. 1985*) and Canine adenovirus type-1 (*CAV-1 Abbassia 2002*) (*Khodeir, et al. 2003, II*) propagated on Vero cell line were used for preparation of attenuated CD; CP; and CH vaccines and inactivated rabies as well as used in serum neutralization test.

1.2 Virulent strains:

Standard Synder Hill virulent CDV propagated on chicken embryonated eggs (*Gillespie and Richard 1956*), virulent strain of CPV propagated in NLFK" Norden laboratory Feline kidney cell culture (*Parrish, et al. 1985*) and virulent strain of the local isolate of CAV-1 (*Khodeir, et al. 2003, I*) were used for challenge of vaccinated dogs.

Both of vaccinal and virulent virus strains were supplied by the Dept of Pet Animal Vaccine Research; Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo.

2- Virus titration:

Rabies, CDV, CPV₃₉ and CAV-1 were titrated in the corresponding cell culture using the micro-titer technique according to (*Trimarchiet al. 1996*) and the virus titer was calculated according to (*Reed and Menuch 1938*).

3- Vaccine preparation:

3.1- Single vaccines:

Single attenuated cell culture vaccine rabies, CDV; CPV₃₉ and CAV-1 vaccines were prepared according to (*Lawson, et al. 1989; Guirguis, 1991; Koteb, 1994 and Khodeir, et al. 2003, II*) respectively.

3.2- Attenuated tetra dog vaccine baits:

The attenuated rabies; CDV; CPV₃₉ and CAV-1 vaccines were mixed together in a manner that it is sure that the final produced dose of the product contains the protective dose of each vaccine which was previously determined and supported by (*British Pharmacopoeia, 1990 and European pharmacopeia, 2001*). Fish cake meals of a cylinder-shaped of about 4 inches long were injected with only one type of previously prepared vaccines (to prepare single vaccine baits) according to (*Baer, et al. 1989 and Edris, et al. 2001*) Such baits were found to be attractive for dogs (*Joseph, et al. 2003*) and they were administrated after 24 hours starvation.

3.3- Tetra dog vaccine for parental use:

The lyophilized CDV, CPV₃₉ and CAV-1 vaccine with the inactivated rabies vaccine which is used as a diluents prepared according to (*Koteb, and Daoud, 2004*).

4- Quality control tests:

The prepared vaccines were subjected to examination of their freedom from foreign contaminants, safety and potency following the recommendations of the (*European pharmacopeia, 2001and FAO, 1994*).

5- Dogs:

Forty six native puppies of about three months age were found to be free from antibodies against rabies, CD, CP and ICH as screened by SNT. They were also free from external and internal parasites. They housed under hygienic measures in special Kennels. These puppies were divided into seven groups as follow:

- The first four groups (four puppies) were vaccinated with one type of single attenuated vaccines in baits; these puppies were starved 24 hours before bait administration.
- The fifth group (10 puppies) was vaccinated with the attenuated tetra dog vaccine in baits.
- The sixth group (10 puppies) was vaccinated with the tetra dog vaccine parentally (S/C).
- The seventh group (10 puppies) was kept without vaccination as a control group.

Serum samples were obtained from all animal groups at 0, 14 and 30 days post vaccination and at 90 days later. Serum samples were obtained only from puppies which didn't subject to challenge.

6- Challenge test:

Each of puppy's groups vaccinated with CD, CP and ICH were divided into two subgroups, where one subgroup was challenged against the corresponding virus, while the 2nd subgroup was kept to follow up their immune levels. The challenge against the three viruses was carried out through the oronasal rout according to (*Gillespie and Richard 1956; Parrish, et al. 1985 and Khodeir, et al. 2003, II*)

7- Serum neutralization test (SNT):

SNT was carried out to estimate the titer of neutralization antibodies in vaccinated puppies using the micro-titer technique according to (*Bass, et al. 1982*).

RESULTS AND DISCUSSION

Table (1): Titer and doses of the used viruses

Virus	Virus titer	The used dose
ERA strain of rabies	$10^{7.4} \log_{10}$ TCID ₅₀ /ml (before inactivation)	The vaccine dose is 2ml for dog
Rockborn CD vaccine virus	$10^7 \log_{10}$ TCID ₅₀ /ml	$10^3 \log_{10}$ TCID ₅₀ / dog
Standard Synder Hill CDV	$10^5 \log_{10}$ EID ₅₀ /ml	$10^5 \log_{10}$ EID ₅₀ / dog for challenge test
CP vaccine virus	$10^{7.5} \log_{10}$ TCID ₅₀ /ml	$10^3 \log_{10}$ TCID ₅₀ /dog for challenge test
Virulent CP virus	$10^{3.8} \log_{10}$ TCID ₅₀ /ml	$10^5 \log_{10}$ TCID ₅₀ /dog for challenge test
CAV-1vaccine virus	$10^{6.8} \log_{10}$ TCID ₅₀ /ml	$10^3 \log_{10}$ TCID ₅₀ /dog for challenge test
Virulent CAV-1	$10^{5.3} \log_{10}$ TCID ₅₀ /ml	$10^5 \log_{10}$ TCID ₅₀ /dog for challenge test

Table (2): Potency of the prepared vaccines

Tested vaccine	Number of vaccinated puppies	Used Challenge virus	Protection % (S/V)*	Post challenge clinical signs
Single CD baits	2	CDV	100%	No clinical signs
Single CP baits	2	CPV		
Single CH baits	2	CHV		
Tetra dog baits	2	CDV		
	2	CPV		
	2	CHV		
S/C tetra dog vaccine	2	CDV		
	2	CPV		
	2	CHV		
Unvaccinated control puppies	2	CDV		
	2	CPV	0	Fever; enteritis; depression and death after 7 days
	2	CHV	0	Fever; depression; ocular opacity and death after 10 days

Table (3): Serum neutralizing antibody titers in different vaccinated puppy groups

Used vaccine	Mean serum neutralizing antibody titer (log ₁₀)/ days post vaccination			
	14DPV*	21DPV	30DPV	90DPV
S/C single rabies vaccine	0.9	1.15	1.35	1.8
Single rabies baits	0.9	1.2	1.65	1.8
Rabies in tetra dog baits	0.975	1.275	1.575	1.875
Single S/C CD vaccine	1.2	1.35	1.8	1.95
Single CD baits	1.05	1.35	1.65	1.8
CD in tetra dog baits	0.975	1.275	1.725	2.025
Single S/C CP vaccine	1.50	1.8	2.4	2.7
Single CP baits	1.35	1.65	2.4	2.55
CP in tetra dog baits	1.425	1.725	2.25	2.625
Single S/C CHI vaccine	1.2	1.65	1.95	2.4
Single CHI baits	0.9	1.20	1.80	2.1
CHI in tetra dog baits	0.96	1.275	1.95	2.175

*DPV= days post vaccination.

The permissible protection level of vaccine is:

Rabies: 0.5; canine distemper: 1.7; canine parvo: 1.2 and for canine infectious hepatitis: 1: 10

DISCOUSION

Rabies, canine distemper, canine parvo and infectious canine hepatitis are common contagious viral diseases of dogs and many other carnivores, these diseases posing a several threat to the population dynamics of domestic and wild carnivores, as well as endangering carnivore conservation (*Greene, 2006*). So vaccine based prophylaxis has greatly helped to keep these infectious diseases under control (*Wangx, et al. 2008*).

Since the oral route of the delivery can stimulate strong protective responses on mucous membranes and in the circulation (*Holmgren, 1991 and, Cui, et al. 1991*) and it may circumvent the potential problem in the limitation of the volume of the multi component vaccines when given parentally. In addition oral vaccination may be an effective strategy for immunizing animals that contain maternal antibodies (*Noemi, et al. 1995*).

Therefore, in this study, dogs were vaccinated by oral delivery core vaccines using baits. The prepared baits were found to be attractive to puppies, with suitable size and safe where the puppies ate them completely and remain healthy without showing abnormal clinical signs in agreement with the conditions recommended for oral bait vaccines by the (*WHO, 2007*). Several type of modified live virus rabies have been proposed for the oral immunization of animals in the past 20 years, however, only six MLV vaccines have proved suitable for use in the field, all of them are derivatives of the original SAD virus (*Blancou and Meslin 1996*), ERA strain is one of them; it was used to vaccinate puppies against rabies either per os (baits) or parentally (S/C).

The potency of the attenuated rabies vaccine carried out by the titration of virus which is reliable indicators of vaccine efficacy according to (*OIE, 2008*) and by measuring the neutralizing rabies antibodies in the sera of vaccinated puppies according to the (*WHO, 1985*) 0.5 IU is the minimum measurable rabies antibodies titer considered to represent a level of immunity that correlates with the ability to protect against rabies infection.

Regarding *table (1)*, the titer of the rabies virus is $10^{7.4}$ TCID₅₀/ml 2 ml which is efficient to induce virus neutralizing antibodies in vaccinated puppies according to (*Lawson, et al. 1989*).

Table (3) showed the sero-conversion using virus neutralizing test which revealed that all puppy groups vaccinated with rabies vaccine exhibited good levels of specific rabies antibodies at the 14th day after vaccination and it was noticed that vaccination with rabies alone or with other vaccines did not interfere with the immune response of the puppies and also it was noticed that the live attenuated rabies vaccine administrated through baits induced higher levels of neutralizing antibodies specially at the 14th day after vaccination. These results agree with those obtained by (*Hafliger, et al. 1982; Kerrimbov, et al. 1985; Blancou, et al 1990 and Edris, et al. 2001*).

Concerning the potency test of CDV, CPV and CAV-1, *table (2)* revealed that all vaccinated puppies exhibited a 100 % protection against challenge with the corresponding virulent strain, while non vaccinated dogs showed typical symptoms of CD (ocular and nasal discharge, fever then recovered), CP (fever enteritis depression and death 7 days later) and CH (Fever; depression; ocular opacity and death after 10 days). These symptoms were similar to those mentioned by (*Greene, 2006*).

The results of the sero-conversion (SNT) in *table (3)* were correlated to the results of potency test where it revealed that detectable levels of antibodies appear in the first sample 14 days after vaccination and reached good levels after one month of vaccination in all groups of vaccinated dogs. These levels of antibodies protect animals against challenge with virulent viruses and came in agreement with (*CFR ,1997*) which recommended that serum neutralizing antibody titer should not be less than 1:50 ($1.7 \log_{10}$) for the CD ; 1: 16 ($1.2 \log 10$) for CP and 1 : 10 for CAV-1.

The obtained results also agreed with those of (*Sprino, and Harris, 1983, Guirguis, 1991, Miyamoto, et al. 1995, Khodeir, et al. 1998 and Koteb and Daoud, 2004*) who reported that dogs were considered immune to CD if their antibody titer was higher than 1:30 while CP titer of 1 : 8 is protective against clinical disease and intestinal replication of virulent virus as mentioned by (*Ruth and Emary, 1981 and Ackerman, et al. 1983*) . For CAV-1, any increase in serum neutralizing antibody titer could be considered protective as mentioned by (*Cooper, et al. 1991 and CastroAnd Heuschele, 1992*).

A lot of stray dogs and cats cause serious threat of rabies and other zoonotic disease transmission, so from the present obtained results; it could be concluded that the oral delivery bait vaccines could help controlling of such zoonotic diseases by immunization of such animals rather than their eradication.

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تحصين الأجرأو باللقاحات الأساسية عن طريق الفم (لقاحات الديستمبر والبارفو والإلتهاب الكبدى والسعار)

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معهد بحوث الأمصال واللقاحات البيطرية

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تم خلال هذه الدراسة تحضير أربع لقاحات نسيجية ضد كل من الديستمبر والبارفو والإلتهاب الكبدى والسعار بصور أحادية حيث تم تحضير اللقاحات الأولى كلقاحات حية مستضعفة فى خلايا كلى القرد الأخضر الأفريقى بينما تم تحضير لقاح السعار فى خلايا اليربوع السورى الذهبى كلقاح حي مستضعف ومثبط. وقد تم تحصين مجموعات مختلفة من الأجرأو بكل لقاح على حدى وفى صورة مركبة (لقاح رباعى) عن طريق الحقن تحت الجلد. كما تم تحضير طعوم من كيك الأسماك لكل لقاح على حدى وإيضا فى صورة مركبة حيث تم تحصين مجموعات أخرى من الأجرأو بهذه الطعوم. وقد تبين أن كل من اللقاحات الأحادية والمركبة آمنة وفعالة سواء تم التحصين التحصين بها بصورة أحادية أو مركبة عن طريق الحقن أو عن طريق الطعوم. وقد أوضحت نتائج اختبار المصل المتعادل أن الحيوانات المحصنة تكتسب أجساما مناعية نوعية ضد كل من الفيروسات المستخدمة فى تحضير اللقاحات دون تداخل سلبى بينها على استجابة الحيوانات المناعية عند استخدامها بصور مركبة كما كانت نسبة الحماية 100% عند إجراء اختبارات التحدى بالفيروسات الضارية بينما أظهرت الحيوانات غير المحصنة أعراضا مرضية مميزة لكل مرض. وعلى ذلك يمكن القول بأن استخدام الطعوم لتحصين

الكلاب آمن وفعال يوفر الوقت والجهد ويتغلب على بعض المشاكل التي قد تصادف التحصين عن طريق اللقاحات.