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Potency of inactivated rabies vaccine with live attenuated canine distemper and canine parvovirus vaccines

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ABSTRACT

Canine parvovirus (CPV), canine distemper virus (CDV) and rabies are viruses that cause life-threatening infections in dogs and have a large effect on the canine population. This work aims to investigate the effect of inactivated rabies vaccine on the immune response of vaccinated puppies with live attenuated CD and CP vaccines. Antibody response of puppies vaccinated with CD, CP and rabies vaccines either in single or simultaneous administration was evaluated through vaccination of different puppy groups and monitoring the levels of induced antibodies in their sera through application of SNT and indirect ELISA. The two serological assays revealed that the simultaneous vaccination of puppies with CD or CP vaccines with rabies vaccine induced higher levels of CD or CP antibodies 256 by SNT and 3.5 log10 by ELISA) than in case of Puppies vaccinated with live CD or live CP vaccines alone. So, it could be recommended to vaccinate puppies simultaneously with attenuated CD and inactivated rabies vaccines, or attenuated CP and inactivated rabies vaccines or with the three vaccines at one shot.

1. INTRODUCTION

Rabies, a zoonotic disease affecting the central nervous system (CNS), causes acute and fatal encephalitis in its mammalian hosts. The rabies virus (RABV) is the etiologic agent; it is a neurotropic RNA virus that belongs to the order Mononegavirales, family Rhabdoviridae, genus Lyssavirus. (**Fauquet et al, 2005**). Various mammals, particularly those of the orders Carnivora and Chiroptera, serve as reservoirs for RABV in various parts of the world (**Rupprecht, 2002**). Infected saliva is deposited in bite wounds, skin scratches, and breached mucous membranes, which leads to transmission.

Canine distemper virus is classified in the genus Morbillivirus within the family Paramyxoviridae and has an unsegmented, negative-sense, single-stranded, 15.7-kb RNA genome and an enveloped virus particle that is 150 to 300 nm in diameter (**Murphy, 1999**). CDV is one of the zoonotic diseases that is likely to affect humans, particularly children. A runny nose, vomiting, and diarrhoea, dehydration, excessive salivation, coughing and/or laboured breathing, loss of appetite, and weight are all common symptoms of canine distemper. Localized involuntary twitching of muscles or groups of muscles, seizures with salivation and jaw movements, or "chewing-gum fits," or more appropriately, "distemper myoclonus," are all central nervous system symptoms. As the condition progresses the seizures worsen and advance to grand mal convulsions followed by death of the animal (**Creevy, 2013**). To prevent canine distemper, puppies should be vaccinated at 6-8 weeks of age and then given a "booster shot" every 2-4 weeks until they are 16 weeks old (**MAR VISTA Vet, 2012**) .

Canine parvovirus (CPV2) is the most dangerous viral etiology in dogs that causes gastroenteritis. Canine parvovirus 2 is a highly contagious and often fatal disease that causes acute hemorrhagic enteritis and myocarditis in dogs (Decaro et al., 2005). The virus was given the name CPV-2 to distinguish it from a closely related canine parvovirus known as CPV-1 or minute virus of canine (MVC) (**Carmichael, 2005**). The vaccine, which is based on the original antigenic type CPV-2, has been shown to protect dogs against infection with the new antigenic types (CPV-2a/2b) (**Yule et al., 1997**). Attenuated CPV vaccines provided excellent protection and longer immunity (**Spibey et al., 2008**) . The use of specific antibody response potent vaccines is essential in protecting dogs and limiting the spread of these diseases. These vaccines can be given in single or combined doses. The use of combined vaccines saves time and effort by protecting dogs against multiple viruses in a single injection (**Cooper et al, 1995; Khodeir et al; 1998; Saleh et al, 2002 and Decaro et al, 2008**)

The present work aims to evaluate the of dog antibody response vaccinated with inactivated rabies vaccine and live attenuated canine distemper or canine parvo vaccines to determine the best vaccination schedule providing dogs with the highest levels of immunity against these diseases.

2. MATERIAL AND METHODS

2.1.Ethical approval

Care and use of the animals were approved by the Medical and Veterinary Research Ethics Committee at the National Research Centre in Egypt (No,.20/053).

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2.2 Virus strain and cell culture

2.2.1 .Viruses

The Evelyn Rokitnicki Abelseth (ERA) strain of rabies virus was adapted in BHK-21 cell culture and had a titer of 107 TCID50/ml (Edries, 1994), Vero cell culture adapted canine distemper virus Snyder Hill strain with a titer of 105 TCID50 /ml and canine (Guirguis, 1991) and canine parvovirus type 2a with a titer of 105.5 TCID50 /ml (El-Gendy,2018) were supplied by the Department of Pet Animal Vaccine Research (DPAVR), Veterinary Serum and Vaccine Research Institute (VSVRI) Abassia Cairo and used in vaccine preparations and serological tests

2.2.2.Cell culture

Baby hamster kidney cell line (BHK21), African green monkey kidney cell line (Vero), and Madin-Darby canine kidney (MDCK) cell lines were supplied by VSVRI for the propagation of CP and rabies viruses and CD virus, respectively, for vaccine preparations and serum neutralization tests.

2.3.Animals

2.3.1.Puppies: Twenty one native breed puppies of 3- 4 months age were found to be free from CD; CP and rabies antibodies as screened by SNT and subjected to the present experimental work .

2.3.2.Mice: Twenty eight Albino Swiss weaned mice supplied by VSVRI were divided into 4 groups (7 mice/group) used to test the safety of CD; CP and rabies vaccines in the first 3 groups leaving group 4 as control without inoculation according to (**WHO, 1973**)

2.4 Preparation of inactivated rabies vaccine

ERA virus strain of rabies was propagated in BHK21 cell culture and a virus suspension was prepared with a titer of 6.5log10 TCID50/ml then inactivated with binary ethylene amine according to(Edreis ,2017) then Montanide pet gel A was added at the ratio of 10% as adjuvant according to the manufacturer directions

2.5.Preparation of attenuated CP and CD vaccine

To prepare monovalent live attenuated vaccines of (either CD or CP), stabilizer composed of 5% lactalbumin hydrolysate and 2.5% sucrose was added to the titrated and sterility tested virus suspension in the ratio of 1:1 then dispensed in neutral sterile vials (2.5ml/vial) and subjected to freeze-drying (lyophilization) process according to (Guirguis, 1991 and Koteb, 1994).Then vaccines undergo-Freeze-drying process by Teflon lyophilize apparatus according to(Wang and Zhang, 2007)

2.6.vaccination protocol

Seven groups of native breed puppies (3-4 months of age) free from CD, CP and rabies antibodies as screened by SNT were used to fulfillment the object of the present study subjected to the experimental work as follow where each group includes 3 puppies:

Group-1 vaccinated with live CD vaccine using a dose of 10 3 TCID 50 of CD virus/ puppy inoculated subcutaneously

Group-2 vaccinated with live Cp vaccine using a dose of 10 3 TCID 50 of Cp virus/ puppy inoculated subcutaneously

Group-3 vaccinated with 1ml inactivated rabies vaccine / puppy inoculated subcutaneously.

Group-4vaccinated with a dose of attenuated CD vaccine dissolved in 1 ml inactivated rabies vaccine / puppy inoculated subcutaneously

Group-5 vaccinated with a dose of attenuated CP vaccine dissolved in 1 ml inactivated rabies vaccine / puppy inoculated subcutaneously

Group-6 vaccinated with a dose of live CD vaccine and a dose of live CP vaccine dissolved in 1 ml inactivated rabies vaccine / puppy inoculated subcutaneously

Group-7 was kept without vaccination as control

Serum samples were obtained from all puppies on week intervals up to 4 weeks post vaccination then on 2 months intervals up to 12 months later for monitoring of induced antibodies using serum neutralization test

2.7 .Serum neutralization test(SNT)

SNT was carried out to estimate rabies neutralizing antibodies in serum of test animals as described by **(Yoneda et al., 2008)** and the antibody titer was determined as the reciprocal of the final serum dilution which neutralized and inhibited the appearance of the cytopathic effect (CPE) of 100 TCID50 of rabies virus according to **(Singh et al ., 1967)** .

2.8 .Indirct Enzyme linked Immunosorbent Assay ELISA

According to the bombinated methods of (Volleret al., 1976) indirect ELISA was carried out for monitoring of CD, CP and rabies induced in vaccinated puppies using anti-dog immunoglobulin [IgG whole molecule] conjugated with Horse Radish Peroxidase (HRP)] obtained from Sigma Chemical Company (USA).

3. RESULTS

It was confirmed that both of single live CD and CP and inactivated rabies vaccines were free from any contaminants as tested on specific media for aerobic and anaerobic bacteria; fungi and mycoplasma. In addition, safety testing of these vaccines was established in mice and puppies showing no local or general abnormal post inoculation manifestation .

Serum neutralization test and indirect ELISA carried out for following up CD and CP antibodies in vaccinated puppies (tables 1, 2, 3and 4) showed that such levels were higher in puppies vaccinated with CD or CP live vaccines with inactivated Rabies vaccine or with the three vaccines (256 by SNT and 3.5 log10 by ELISA) than in vaccinated puppies with CD vaccine alone (128 by SNT and 2.5 log10 by ELISA) by the fourth week post vaccination. These antibody titers were stable up to 12 months later .On the other side vaccinated puppies with the inactivated rabies vaccine either singly or simultaneously with CD or CP or with the three vaccines, showed the same immune response behavior exhibited the same specific rabies antibody titers (128 by SNT and 5.5-6 log10 by ELISA) as shown in tables(5,6)

4. DISCUSSION

Canine distemper (CD), Canine parvo and rabies infections represent three of the most dangerous viral diseases of dogs that cause great losses among dog populations. Puppy immunization against the three diseases is considered the corner stone to control them and minimize their spread through efficient vaccination programs with potent vaccines. The present study evaluate the efficacy of puppy vaccination with live CD and CP vaccines and inactivated rabies vaccine either in single or simultaneous vaccinations . The obtained results revealed that all prepared vaccines (live CD and CP and inactivated rabies vaccines) were free from aerobic and anaerobic bacteria; fungi and mycoplasma and safe inducing no abnormal local or systemic post vaccinal reactions in agreement with the recommendation of (WHO,1973).

Table (1): Mean CD neutralizing antibody titers in puppies vaccinated by inactivated rabies vaccine with live attenuated canine distemper or canine parvovirus vaccines

Puppy Groups	Mean CD serum neutralizing antibody titer*										
	0 time	1WPV **	2 WPV	3 WPV	4 WPV	2MPV ***	4 MPV	6 MPV	8 MPV	10 MPV	12 MPV
G-1	0	4	8	16	64	← 128 →					
G-4	0	16	32	64	128	← 256 →					
G-6	0	16	32	64	128	← 256 →					
G-7	0	0	0	0	0	0	0	0	0	0	0

The protective titer = 32
*CD serum neutralizing antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited the CPE of $100TCID_{50}$ of CD virus
**WPV= weeks post vaccination ***MPV= months post vaccination
G-1 = puppies vaccinated with live CD vaccine alone
G-4 = puppies vaccinated with live CD vaccine and inactivated rabies vaccine
G-6 = puppies vaccinated simultaneously with the three vaccines
G-7 = non vaccinated puppy's control

Table (2): Mean CD ELISA antibody titers in puppies vaccinated by inactivated rabies vaccine with live attenuated canine distemper or canine parvovirus vaccines

Puppy Groups	Mean CD ELISA antibody titer (log10)										
	0 time	1WPV **	2 WPV	3 WPV	4 WPV	2MPV ***	4 MPV	6 MPV	8 MPV	10 MPV	12 MPV
G-1	0.02	0.90	1.33	1.95	2.23	← 2.40-2.50 →					
G-4	0.03	1.80	2.45	2.90	3.10	← 3.0 -3.5 →					
G-6	0.01	1.90	2.5	2.83	3.0	← 3.0 – 3.5 →					
G-7	0.21	0.22	0.24	0.04	0.03	0.02	0.28	0.21	0.30	0.22	0.24

The protective ELISA titer = 1.0 or more
**WPV= weeks post vaccination ***MPV= months post vaccination
G-1 = puppies vaccinated with live CD vaccine alone
G-4 = puppies vaccinated simultaneously with live CD vaccine and inactivated rabies vaccine
G-6 = puppies vaccinated simultaneously with the three vaccines
G-7 = non vaccinated puppy's control

Table (3): Mean CP serum neutralizing antibody titers in puppies vaccinated by inactivated rabies vaccine with live attenuated canine distemper or canine parvovirus vaccines

Puppy Groups	Mean Cp serum neutralizing antibody titer*										
	0 time	1WPV **	2 WPV	3 WPV	4 WPV	2MPV ***	4 MPV	6 MPV	8 MPV	10 MPV	12 MPV
G-2	0	8	16	32	64	← 128 →					
G-5	0	16	32	64	128	← 256 →					
G-6	0	8	16	64	128	← 256 →					
G-7	0	0	0	0	0	0	0	0	0	0	0

The protective CP-SNT= 16
*Cp serum neutralizing antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited the CPE of $100TCID_{50}$ of CP virus
**WPV= weeks post vaccination ***MPV= months post vaccination
G-2 = puppies vaccinated with live CP vaccine alone
G-5 = puppies vaccinated simultaneously with live Cp vaccine and inactivated rabies vaccine
G-6 = puppies vaccinated simultaneously with the three vaccines
G-7 = non vaccinated puppy's control

Table (4): Mean CP ELISA antibody titers in puppies vaccinated by inactivated rabies vaccine with live attenuated canine distemper or canine parvovirus vaccines

Puppy Groups	Mean Cp ELISA antibody titer (log10)										
	0 time	1WPV **	2 WPV	3 WPV	4 WPV	2MPV ***	4 MPV	6 MPV	8 MPV	10 MPV	12 MPV
G-2	0.40	0.70	0.93	1.01	2.0	← 2.5- 3.5 →					
G-5	0.22	2.8	3.0	3.20	3.5	← 3.2- 3.5 →					
G-6	0.31	2.9	3.10	2.90	3.4	← 3.0 -3.5 →					
G-7	0.20	0.11	0.21	0.20	0.21	0.00	0.20	0.30	0.20	0.22	0.22

The protective ELISA = 1.0 or more
**WPV= weeks post vaccination ***MPV= months post vaccination
G-2 = puppies vaccinated with live CP vaccine alone
G-5 = puppies vaccinated simultaneously with live CP vaccine and inactivated rabies vaccine
G-6 = puppies vaccinated simultaneously with the three vaccines
G-7 = non vaccinated puppy's control

Table (5): Mean rabies serum neutralizing antibody titer in puppies vaccinated by inactivated rabies vaccine with live attenuated canine distemper or canine parvovirus vaccines s

Puppy Groups	Mean rabies serum neutralizing antibody titer*										
	0 time	1WPV **	2 WPV	3 WPV	4 WPV	2MPV ***	4 MPV	6 MPV	8 MPV	10 MPV	12 MPV
G-3	0	4	16	32	64	← 128 →					
G-4	0	2	8	16	64	← 128 →					
G-5	0	4	8	32	64	← 128 →					
G-6	0	4	16	32	64	← 128 →					
G-7	0	0	0	0	0	0	0	0	0	0	0

The protective rabies SNT= 8

*CD serum neutralizing antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100$TCID_{50}$ of rabies virus

**WPV= week post vaccination ***MPV= month post vaccination

G-3 = puppies vaccinated with inactivated rabies vaccine alone

G-4 = puppies vaccinated simultaneously with live CD vaccine and inactivated rabies vaccine

G-5 = puppies vaccinated simultaneously with live CP vaccine and inactivated rabies vaccine

G-6 = puppies vaccinated simultaneously with the three vaccines

G-7 = non vaccinated puppy's control

Table (6): Mean Rabies ELISA antibody titers in puppies vaccinated by inactivated rabies vaccine with live attenuated canine distemper or canine parvovirus vaccines

Puppy Groups	Mean CD ELISA antibody titer (log10)										
	0 time	1WPV **	2 WPV	3 WPV	4 WPV	2MPV ***	4 MPV	6 MPV	8 MPV	10 MPV	12 MPV
G-3	0.11	1.5	2.5	3.5	5.0	← 5.5-6.0 →					
G-4	0.20	1.0	2.0	2.5	4.0	← 5.0-5.5 →					
G-5	0.30	0.9	2.0	2.3	3.9	← 5.0-5.6 →					
G-6	0.22	1.3	2.5	2.9	4.5	← 5.6- 5.9 →					
G-7	0.20	0.11	0.21	0.20	0.21	0.00	0.20	0.30	0.20	0.22	0.22

The protective ELISA titer= 1.0 or more

**WPV= week post vaccination ***MPV= month post vaccination

G-3 = puppies vaccinated with inactivated rabies vaccine alone

G-4 = puppies vaccinated simultaneously with live CD vaccine and inactivated rabies vaccine

G-5 = puppies vaccinated simultaneously with live CP vaccine and inactivated rabies vaccine

G-6 = puppies vaccinated simultaneously with the three vaccines

G-7 = non vaccinated puppy's control

The levels of CD antibodies recorded in tables (2&3) in animals vaccinated with live CD vaccine alone or with 2012) recommended that serum neutralizing CD antibody titer should not be less than 1:50 (1.7 log10) for the CD. The obtained results also agreed with (Guirguis , 1991); Miyamoto et al., 1995; Khodier et al. 1998 and Aly and Salama, 2005) who reported that dogs were considered immune to canine distemper if their antibody titer was higher than 30 .

It was found that CP antibody titers estimated by SNT and ELISA tabulated in tables (5&6) were found to be with high protective levels induced by live CP vaccine either in single vaccination or with inactivated rabies vaccine. These levels of CP antibodies appear to be higher than the recommended protective levels where titer of 8 is protective against clinical disease and intestinal replication of virulent virus as mentioned by (Ruth and Emery, 1981)on the other side, (Fiscus et al., 1985) consider neutralizing titer of 16 is protective. In addition, similar findings and recommendations were obtained by (Khodier et al., 1998; Koteb et al., 1998; Saleh et al., 2002; Koteb and Douad, 2004 and Aly and Salama, 2005). Also, coming in a parallel manner to those reported by (Spibey et al., 2008) who stated that attenuated CPV vaccines provided excellent protection .

Vaccination of dogs with a single dose of the inactivated cell culture rabies vaccine resulted in induction of specific rabies neutralizing antibodies from the first week post vaccination with a mean titer of 3.33 that increased gradually to reach its peak (64-128) by the 4th week later then still unchanged till the 16th week. ELIZA results inactivated rabies vaccine or with live CP and inactivated rabies vaccine came in agreement with (Marvista Vet, showed similar behavior of its titers as SNT where the obtained mean titers were 7- 6log2 by the 4th week. These results came to be parallel to and confirmed by the findings of (Khodier ,1999; Khodeir and Daoud , 2008; Albehwar , 2009 and Edries et al,.2017) who obtained similar results and stated that the cell culture inactivated rabies vaccine is safe for all animal species and clarified that the protective neutralizing antibody titer should not be less than 1:5

CD and CP antibody titers increase were earlier in animals inoculated with CD and CP live vaccine with inactivated rabies vaccine than in vaccinated ones with CP vaccine alone the peak antibody titer was the same in all groups (256) by the 3rd to the 4th month post vaccination and retained at this level up to 12 months post vaccination as tabulated in table (2,3,4&5). These findings could be explained on the fact that Montanide Pet Gel A in rabies vaccine induces superior antibodies production higher than other adjuvants where it based on a dispersion of a high molecular weight polyacrylic polymer (Vialle, 2010). Also, excellent protection and longer immunity and could be attributed to the presence of rabies vaccine adjuvant (Monatide pet gel A) which has an immunomodulator effect (Edries et al,2017)

5-**CONCLUSION**

The obtained results, recommended vaccination of puppies with live CD or CP with the inactivated rabies vaccine adjuvanted with Montanide Pet Gel A to provided them higher and long standing immunity.

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