

STUDIES ON SIMULTANEOUS VACCINATION OF DOGS WITH SOME PET ANIMAL VACCINES

F.K HAMODA* ; S.B ELGALLAD** ; ATTYAT M.KOTEB** ; S. M. EDRIES** ,
AMANI A. SALEH** and W.I. GUIRGIS **

* Anim Med Dept, Fac Vet Med, Zagazig University Benha Branch, Egypt.

**Department of Pet animal vaccine research, Veterinary serum and vaccine Research Institute, Abbasia, Cairo.Egypt.

Received: 17.8.2000.

Accepted: 9.10.2000.

SUMMARY

This investigation was applied on 55 puppies of 2-4 months age to evaluate the efficacy of cell culture live attenuated canine parvo virus and canine distemper vaccines and inactivated cell culture rabies virus vaccine used singly or simultaneously. The efficacy of these vaccines was determined by estimating the antibodies titer using serum neutralization and haemagglutination inhibition tests in puppies sera and the challenge of vaccinated animals with virulent strains of parvo and canine distemper viruses.

All vaccinated animals did not show any signs of illness, also neutralizing and haemagglutination inhibition antibodies were detected in puppies sera from one week post vaccination (1WPV) and reached to a peak level at 3WPV. This level of antibodies was nearly the same in both animal groups vaccinated singly or simultaneously. After

challenge, the vaccinated animals were not exhibited clinical signs related to either parvo virus or canine distemper virus infection while the challenged nonvaccinated animals revealed typical clinical manifestation of canine distemper and parvo virus infection. The challenge against rabies did not done to avoid its hazard to workers

Finally it could be concluded that, simultaneous injection of the above vaccines protected puppies safety at the same time.

INTRODUCTION

Dogs play a vital role in research and security as in policy services. Many of canine diseases have medical importance as zoonotic diseases, so dogs play an important role in epidemiology and control of these diseases.

Canine parvo virus infection (CP), canine distemper (CD) and rabies are endemic viral diseases in Egypt since long time (Bucci et al., 1982, and Bayoumi et al., 1985). Suspicious that CDV might have a zoonotic implication as the human infection might be associated with multiple sclerosis (Gaskell and Bennett., 1996). The public health risk of rabies throughout the world was documented and the domestic dogs were responsible for most human exposure and up to 98 % of human fatalities recorded due to rabies (WHO., 1992). Moreover CP might have an immunosuppressive effect in some dogs as reported by Krakowa et al., (1982) and Kesel and Neil (1983), also CDV might have the same phenomena (Gaskell and Bennett., 1996).

Veterinary vaccinology is a very interesting and rapidly developing field, in fact veterinary vaccines are not only used for prevention of infectious diseases in the animal health sector but also help to solve problems of public health to reduce detrimental environmental impact of the use of some veterinary drugs and prevent emergence of resistance of micro-organisms or parasites (Pastoret., 1999). The use of inactivated instead of attenuated ones in animal vaccination became compulsory in many countries (Pastoret and Falize., 1999) but the live vaccines have benefits in regard to their potential for engendering an enduring immunity against infection where systemic, local and cell mediated immunity would need to be stimulated to achieve full protection (Carmichael

et al., 1984 and Brunner et al., 1989)

Meanwhile, the increased use of combined or multicomponent vaccines could greatly simplify the prophylactics and control of these diseases affecting pet animals and it could reduce the time, cost of the campaign and make it economically more acceptable. It is difficult to predict the kind of infectious agents to which an animal will be exposed, so multivalent vaccines containing immunogens for most of the common infectious disease have been developed (Anon., 1989).

Worldwide, simultaneous immunization of dogs against CD, CP and other canine diseases was documented by many authors (Carmichael et al., 1983; Ackerman., et al 1983; Churchill., 1987; Green., 1992 and Khodeir et al., 1998). Also dual or concurrent infection with the two viruses could occur (Ducatell., et al 1981). Moreover, It is better to administer several attenuated vaccines simultaneously than to give them 1-4 days apart because of the blocking effect that the first vaccine may have on the second due to interferon production (Greene., 1990).

The locally produced vaccines used for vaccination of Egyptian dogs are living attenuated tissue culture canine parvovirus vaccine (Attyat., 1994), inactivated cell culture rabies vaccine (Edries., 1994) and cell culture living attenuated canine distemper vaccine (Guirguis., 1991). A bivalent living attenuated cell culture vaccine was pre-

pared in Vero cells against canine distemper and canine parvo viruses and this vaccine was found to be safe and induced good immunity in Egyptian dogs (Khodeir et al., 1998)

The aim of this study was to evaluate the efficacy of locally produced living attenuated CD and CP and inactivated rabies vaccines in protection of dogs when used singly or simultaneously with each other. The efficacy of these vaccines was investigated through the estimation of the induced antibodies in puppies sera and the challenge of vaccinated animals with virulent strains of parvo and canine distemper viruses.

MATERIAL AND METHODS

I. Animals :

A-Experimental design and vaccination schedule: Fifty five clinically normal puppies of 2-4 months age were allocated into eight groups :

1-G1, 5 puppies, vaccinated with parvovirus vaccine only.

2-G2, 5 puppies, vaccinated with canine distemper vaccine.

3-G3, 5 puppies, vaccinated with rabies vaccine.

4-G4, 10 puppies, inoculated simultaneously by canine parvovirus and distemper vaccines (5 puppies were used in experimental challenge with canine parvovirus and the other

puppies were used in challenge with canine distemper virus).

5-G5, 5 puppies, injected simultaneously by canine parvovirus and rabies vaccines.

6-G6, 5 puppies, vaccinated simultaneously by canine distemper and rabies vaccines.

7-G7, 10 puppies, vaccinated by canine parvovirus, canine distemper and rabies vaccines (5 puppies were used in experimental challenge with canine parvovirus and the other puppies were used in challenge with canine distemper virus).

8-G8 (10 puppies) were kept non vaccinated as control group (5 puppies for experimental challenge with CP virus and the other 5 puppies for challenge by CD virus).

B-Clinical examination :

All animals under experiment were kept under hygienic measures and received sufficient feed and water. They were exposed to fully clinical examination according to Kelly., (1984) and were put under close observation for three months post vaccination. All animals were found to be clinically normal and free from external and internal parasites after parasitological analysis. The body temperature of both vaccinated and non vaccinated puppies was recorded daily for one week post vaccination and one week post challenge. Also all of them were screened before vaccination and they were found to be free from antibodies against

rabies, CP and CD viruses.

II. Vaccines and vaccination: The vaccines used were living attenuated tissue culture canine parvovirus vaccine (Attyat., 1994 and Attyat et al., 1998), inactivated cell culture rabies vaccine (Edries., 1994) and living attenuated tissue culture canine distemper vaccine (Guirguis., 1991). These vaccines produced by department of pet animal vaccine research, Veterinary serum and vaccine Research Institute, Abbasia, Cairo, each vaccine vial contained a single dose and injected subcutaneous (S/C) / dog.

III. The virulent viruses : Virulent canine parvovirus was kindly supplied by Cornell university while the Synder-Hill strain of canine distemper was supplied by James A Baker, Institute for animal health USA, these viruses were used for the challenge test. The infectivity of these viruses in cell culture was calculated according to the method of Reed and Muench (1938).

IV. Cell cultures : African green monkey cells (Vero) and BHK21 were used for preparation of the vaccines and in serology.

V. Sampling : Blood on anticoagulant was taken for estimation of leukocytes count and other blood samples were collected for sera separation from all animals under investigation either before

and at 1, 2 and 3 weeks post vaccination. The sera were stored at -200c until used for serological analysis.

IV. Analytical procedures :

A-Estimation of total leukocytic count : It was applied according to the method of Schalm et al., (1975).

B-Serological tests :

1-Serum neutralization test (SNT) : Microtiter SNT was carried according to Bass, et al. (1982) to detected serum neutralizing antibodies against CPV, RV, CDV in sera of vaccinated puppies.

2-Haemagglutination inhibition test (HI) : It was applied using 1 % porcine red blood cells according to Carmichael., et al. (1980) to determine HI antibody in sera of vaccinated animals.

RESULTS AND DISCUSSION

Canine parvo virus, canine distemper and rabies are infectious diseases of dogs, widely distributed and have highest fatality rate (Appel et al., 1979; Greene., 1990; Nelson and couto., 1992; Appel and Manoli., 1994; Radostits et al., 1995 and Gaskell and Bennett., 1996). In veterinary practice, preventive vaccination is the rule in domestic ani-

imals and seems to be the most appropriate strategy towards eradication of these diseases.

Control of rabies in dog relies principally on the mass immunization of dogs in order to achieve population immunity levels sufficient to inhibit rabies transmission (Perry and wandeler., 1993). Also, preventive vaccination of animals against parvo virus infection and distemper based mainly on using of their vaccines used singly or simultaneously with other canine vaccines and considered as an essential cornerstone in eradication of these diseases. (Greene., 1990 and Gaskell and Bennett., 1996).

A live attenuated egg adapted rabies (Flury low egg passage) vaccine was produced in Egypt but

WHO. (1979) recommended the production of inactivated vaccine, so a locally inactivated rabies vaccine was prepared on BHK21 and was found to be safe and potent (Edries., 1994; Edries et al ., 1998 and Khodeir., 1999), moreover Khodeir., et al. (1998) reported that the prepared CD and CP bivalent vaccine was safe and potent for protection of puppies against CD and CP diseases

In this investigation, all animals vaccinated either singly or simultaneously by CD, CP and rabies vaccines did not show any clinical signs related to CD, CP or rabies. In this respect Durchfel et al (1990) observed neurological signs as grand mal seizures and circling in puppies vaccinated with modified live canine distemper vaccine at 18 days post vaccination.

Table (1): Mean values of total leukocyte in puppies vaccinated with parvo virus vaccine singly or in combination with other vaccines.

Puppy groups	Mean of leukocytic count (10^3 /cumm) post vaccination						
	1	2	3	4	5	6	7
G1 (puppies vaccinated with CPV only)	7.3	7.4	7.2	7.8	7.6	7.8	7.6
G4 (puppies vaccinated with CPV and CDV)	7.2	7.1	6.9	7.9	6.8	7.0	6.9
G5 (puppies vaccinated with CPV and RV)	8.3	8.2	7.8	7.6	7.9	7.9	8.0
G7 (puppies vaccinated with CPV and RV)	8.0	8.1	7.9	8.2	8.5	8.4	8.3
G8 (Unvaccinated control puppies)	7.0	7.1	6.9	7.2	6.8	7.2	6.8

Table (2): The immune response to CPV vaccine in puppies vaccinated with it singly or in combination with other vaccines.

Puppy groups	Antibody titers/weeks post vaccination					
	1W		2W		3W	
	*HIT	**DNT	HIT	SNT	HIT	SNT
G1 (puppies vaccinated with CPV only)	40	16	80	128	320	512
G4 (puppies vaccinated with CPV and CDV)	20	16	80	128	640	1024
G5 (puppies vaccinated with CPV and RV)	40	8	160	64	640	512
G7 (puppies vaccinated with CPV, CDV and RV)	40	16	320	128	640	1024
G8 (Unvaccinated control puppies)	<5	0.0	<5	0.0	<5	0.0

* HIT = Haemagglutination inhibition titer log₂/ml

**SNT = Serum neutralizing antibody titer = the reciprocal of serum dilution which neutralize 100 TCID₅₀ of CP virus in tissue culture.

Table (3): Results of challenge of vaccinated puppies against CP virus

Puppy groups	Appearance of specific clinical signs	Protection %
G1 (puppies vaccinated with CPV only)	No signs	5/5 (100)
G4 (puppies vaccinated with CPV and CDV)	One puppy showed slight signs and recovered within one week.	4/5 (80)
G5 (puppies vaccinated with CPV and RV)	No signs	5/5 (100)
G7 (puppies vaccinated with CPV and RV)	No signs	5/5 (100)
G8 (Unvaccinated control puppies)	Characteristic signs as fever, bloody diarrhea, dehydration and death of all animal's group were observed	0/5 (0.0)

Challenge test was applied after 21 days post vaccination through oro-nasal route using 103.5 TCID₅₀/Puppy.

Table (4): CD Serum neutralizing antibody titer in puppies vaccinated

Puppy groups	Antibody titer*/weeks post vaccination		
	1W	2W	3W
	4	16	64
G2 (puppies vaccinated with CPV only)	4	16	64
G4 (puppies vaccinated with CPV and CDV)	<2	8	32
G6 (puppies vaccinated with CPV and RV)	2	8	64
G7 (puppies vaccinated with CPV, CDV and RV)	<2	4	32
G8 (Unvaccinated control puppies)	0.0	0.0	0.0

* Antibody titer = The reciprocal of serum dilution which neutralize and inhibit the CPE of 100 TCID50 of CDV in tissue culture.

Table (5): Results of challenge of vaccinated puppies against CP virus

Puppy groups	Appearance of specific clinical signs	Protection %
G2 (puppies vaccinated with CPV only)	One puppy showed ocular secretion	4/5 (80)
G4 (puppies vaccinated with CPV and CDV)	No signs	5/5 (100)
G6 (puppies vaccinated with CPV and RV)	No signs	5/5 (100)
G7 (puppies vaccinated with CPV, CDV and RV)	One puppy was died without specific signs	4/5 (80)
G8 (Unvaccinated control puppies)	All puppies revealed fever, ocular-nasal discharges and pustules on the abdominal skin in one puppy.	0/5 (0.0)

Challenge test was applied after 21 days post vaccination using 10⁵ TCID50 of virulent CD virus/Puppy given intranasal.

The body temperatures of investigated animals were within the normal level till one week post vaccination. Results of the total leukocytic count (Table 1), declared no changes in the count in vaccinated puppies. These results indicated and supported the safety of these vaccines when used singly or simultaneously with each others, these findings came in harmony with those obtained by Attyat et al., (1999), Guirguis et al., (1999) and Edries et al., (1998).

Regarding seroconversion of vaccinated animals (Table 2, 4 & 6), it was found that, generally, all vaccinated puppies provoked antibody titer at one week post vaccination and reached a peak level within three weeks post vaccination (3 WPV). The obtained results were similar to that

reported by Bass, et al., (1982), Attyat., (1994); Attyat et al., (1998), Khodeir et al., (1998); Guirguis et al (1999) and Attyat et al., (1999). Also, the above results were agreed with those obtained by Sikes et al (1971) who reported that serum neutralization level of 1:5 or greater was a protective level against rabies infection and less than that was considered negative and Ruth and Emery (1981) who reported that, the protective titer against parvo virus infection by SNT was 8 (0.9 log₁₀) but Fiscus et al., (1985) considered the protective titer of SNT was 16 (1.2 log₁₀), while Carmichael et al., (1983) cited that HI antibody titer > 80 (1.9 log₁₀) was protective one, also dogs were considered immune to canine distemper if their antibody titer was higher than 30 (Gillespie et al., 1958).

Table (6): Rabies serum neutralizing antibody titer in vaccinated puppies.

Puppy groups	Antibody titer*/weeks post vaccination		
	1W	2W	3W
	4	16	64
G3 (puppies vaccinated with RV only)	<2	8	64
G5 (puppies vaccinated with RV and CPV)	2	16	128
G6 (puppies vaccinated with CDV and RV)	2	8	64
G7 (puppies vaccinated with CPV, CDV and RV)	<2	16	128
G8 (Unvaccinated control puppies)	0.0	0.0	0.0

* Antibody titer = The reciprocal of serum dilution which neutralize and inhibit the CPE of 100 TCID₅₀ of CDV in tissue culture.

There was no interference or immunosuppression for CPV vaccine on antibody formation against CD vaccine, these results were documented by previous works of Khan et al., (1983); Churchill., (1987); Cooper et al., (1995) and Khodeir et al (1998) and confirmed by the results of leukocytic estimation. Also there was no adverse effect of rabies vaccine on immune response of vaccinated puppies (Edries., et al 1999). However Brenner et al (1989) concluded that the live modified parvo virus vaccine might have an immunosuppressive property.

Regarding results of challenge test (Table 3 & 5), the study declared that, nearly all vaccinated puppies were withstood challenge with virulent viruses of CP & CD and remained healthy with normal body temperature all over the experimental period while non vaccinated puppies after challenge exhibited specific signs of CD or CP viruses infection. These results were similar to that obtained by Mansi., (1951); Carmichael et al., (1983); Kahn et al., (1983), Guirguis., (1991) and Khodier et al., (1998). However challenge test by virulent street virus of rabies was not carried out to avoid a very dangerous hazard and this was documented by suggestions of Sikes, et al (1971).

With respect to protection post challenge, protection percentage was nearly similar in animals vaccinated singly or simultaneously by one, two or three vaccines and neither CD nor CP had an adverse effect on the immune response to the other

one, these observation were supported by the prior work of Zuffa, et al (1987), Khodeir, et al (1998) and Guirguis, et al (1999).

Finally it could concluded that, the used vaccines were safe and potent and there was no difference in the immune response and rate of protection post challenge when they used singly or simultaneously with each other. So it could be recommended to use the simultaneous vaccination to control CD, CP and rabies in Egypt saving time, efforts and cost.

REFERENCES

- Ackerman, O.; Stegman, H. and Jaeger, O., (1983) : Simultaneous immunization of dogs against parvovirus, distemper, rabies, contagious hepatitis and leptospirosis. *Blauen Hefte Fur den tierarzt.*, 67 : 302-308.
- Anon, A. (1989) : Canine and feline immunization guidelines. *JAVMA.*,195 (3) : 314-317.
- Appel, M.J.; Scott, F.W. and Carmichael, L.E., (1979) : Isolation and immunization studies of a canine parvo-like virus from dogs with hemorrhagic enteritis. *Vet Rec.*, 105 : 156-159
- Attyat M.Koteb. (1994) : Studies on preparation of canine parvovirus vaccine.Ph.D.Thesis. Microbiology.Fac.Vet.Med.,Cairo University
- Attyat M.Koteb, Zaki, H.Y. and Sami, S.M. (1998) : Studies on production of an inactivated canine parvovirus vaccine. 4 th Vet.Med. Zag. Congress in Hurghada.
- Attyat M.Koteb; Edries, S.M; Habashi, Y.Z and Saber, M.S. (1999) : Duration of immunity in puppies vaccinated

- against canine parvovirus and comparison between the different used serological methods. *J.Egypt. Vet.Med.Ass.*, 59 (2 & 3) : 1151-1165.
- Bass, E.P.; Gill, M.A. and Beckenhauer, W.H. (1982) : Development of a modified live canine origin parvovirus vaccine. *JAVMA.*, 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.
- Brenner, J.; Markus, R.; Klopfer-Orgad, U. and Trainin, Z. (1989) : The possible enhancement of parvovirus vaccination on the mortality rate of diseased dogs. *Zentralbl Veterinarmed.*, 36 (7) : 547-550
- Brunner, G.; Reabel, G.H. and Rist, L. (1989) : Active immunization against canine parvo virus in breeding kennels by using a new live vaccine. *Praktische Tierarzt.*, 70 (3) : 24-36.
- Bucci, T.J.; Botros, A.M. and EL-Molla, M. (1982) : Canine parvovirus infection : A brief review and report on first cases in Egypt. *J.Egypt. Vet. Med. Assoc.*, 42 (1) : 21-25
- Carmichael, L.E.; Joubert, J.C. and Pollock, R.V. (1980) : Haemagglutination by canine parvo virus : Serological studies and diagnostic application. *Am. J.Vet.Res.*, 41 : 784-791
- Carmichael, L.E.; Joubert, J.C. and Pollock, R.V., (1982) : A modified live canine parvovirus : Immune response. *Corn Vet.*, 73 : 13-29
- Carmichael, L.E.; Pollock, R.V. and Joubert, J.C., (1984) : Response of puppies to canine origin parvo virus vaccines. *Mod. Vet. Practice.*, 65 : 99-102.
- 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.
- Ducatell, R.; Burtonboy, G. and Coussement, W., (1981) : Concurrent parvovirus and distemper virus infection in dogs. *Vet Rec.*, 108 : 310-311
- Durchfel, B.; Baumgartner, W.; Herbst, W. and Brahm. (1990) : Vaccine-associated canine distemper infection in a litter of African hunting dogs (*Lycaon pictus*). *Zentralbl Veterinarmed.*, 37 (3) : 203-212
- Edries, S.M. (1994) : Studies on preparation of inactivated tissue culture antirabies vaccines. Ph.D.Thesis. Microbiology.Fac.Vet.Med., Cairo University.
- Edries, S.M; Attyat M.Koteb 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.Koteb; Habashi, Y.Z and EL-Nakshely, S. (1999) : Some studies on preparation of a combined vaccine against rabies and parvo viral diseases. *Alex. J. Vet. SCI.*, 15(3) : 591-599.
- Fiscus, S.A.; Mildbrand, M.M.; Gordorf, C. and Scoll, W., (1985) : Rapid enzyme linked immunosorbent assay for detecting antibodies to canine parvo virus. *Am.J.Vet.Res.*, 46 (4) : 859-863.
- Gaskell, R.M. and Bennett, M., (1996) : Feline and canine infectious diseases. Blackwell Science, Inc, USA
- Gillespie, J.H.; Baker, J.A.; Robson, D. and Barbara, G., (1958) : The immune response of dogs to distemper virus. *Cornell Vet.*, 2 : 101-126
- Greene, C.E. (1992) : Infectious diseases of the dog and cat. W.B. Saunders Company, Philadelphia, USA
- Guirguis, W.I. (1991) : Trials for preparation of a vaccine against canine distemper. Ph.D.Thesis Microbiology.Fac.Vet.Med., Cairo University

- Guirguis, W.I.; Khodeir, M.H. and Habashi, Y.Z. (1999) : Duration of canine distemper immune level in vaccinated puppies. *J.Egypt.Vet.Med.Ass.*, 59 (2 & 3) : 1015-1027.
- Kahn, D.E.; Eney, J.B.; Smith, M.J. and Spotts, A.M., (1983) : Safety and efficiency of modified live canine parvo virus vaccine. *Vet. M.S.A. Clinician.*, 78 (11) : 1739-1743.
- Kelly, W.R. (1984) : *Veterinary clinical diagnosis*, 3rd. Ed Baillier Tindall New York
- Kesel, M.L. and Neil, D.H. (1983) : Combined modified living attenuated canine parvo virus vaccine immunosuppression with infective shedding. *Vet M.S.A. Clinician.*, 78 (5) : 687-691.
- 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.Koteb; Guirgis, W.I and Habashi, Y.Z. (1998) : Preparation of a bivalent vaccine against canine distemper and canine parvo viruses. 4 th *Vet.Med. Zag. Congress in Hurghada.*
- Krakowa, S.;Olsen, R.G.; Axthelm, M.K.; Rice, J.and Winters, K. (1982) : Canine parvo virus infection, potentiates canine distemper encephalitis attributable to MLV vaccine. *J. Am.Vet. Med. Assoc.* 180 (2) : 137-139.
- Mansi, w. (1945) : *Bacteriology of distemper*. M.D.Thesis, Foad University., Cairo Egypt
- Nelson, R.W. and couto, C.G., (1992) : *Small animal internal medicine*. Mosby Year book, Inc, USA
- Pastoret, P.P., (1999) : *Veterinary vaccinology*. Acad Sci III., 322 (11) : 967-972
- Pastoret, P.P. and Falize, F., (1999) : *Viral veterinary vaccines*. *Dev Biol Stand.*, 101: 73-78.
- Perry, B.D. and wandeler, A.I., (1993) : The delivery of oral rabies vaccines to dogs : An African perspective. *Ondersteport. J. Vet. Res.*, 60 (4) : 451-457.
- Radostits, O.M.; Blood, D.C and Gay, C.C (1995) : *Veterinary medicine A textbook of the diseases of cattle, sheep, pigs, goats and horses*. 8 th Ed. Baillier Tindall, England.
- Reed, I.J. and Muench, J., (1938) : A simple method of estimating fifty percents. *Am.J.Hyg.*, 27 : 493-497.
- Ruth, D.T. and Emery, J.B., (1981) : Clinical trial of a modified live parvo virus vaccine for dogs. *Vet. Med. Small clinic.*, 76 (6) : 830-832.
- Schalm, O.W.; Jain, N.C. and Corroll, E.J., (1975) : *Veterinary hematology.*, 3rd Ed. Fea and Febiger, Philadelphia
- Sikes, R.K.; Peacock, G.V.; Acha, P.N.; Arko, R.J. and Dierks, R., (1971) : Rabies vaccines : Duration of immunity study in dogs. *JAVMA*. 159 : 1491-1499.
- WHO., (1979) : Expert committee on biological standardization. Requirements of rabies vaccine for human use. *Tech. Rep. Ser.*, 658 : 54-130.
- WHO., (1992) : *World survey of rabies*. No.25.WHO/Rabies 92, 203. Geneva:World Health Organization.
- Zuffa, T Salaj, J. and Bouckova, I., (1987) : Testing the caninipar and canipar-D vaccine against canine parvo virus infection under field condition. *Veterinastovi.*, 33 (8) : 375-377.