The use of animal models other than dogs to test the potency of canine parvo vaccine

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Abstract

An inactivated canine parvo vaccine was inoculated by different doses and methods "subcutaneous and intraperitoneal" in hamster, Guinea pigs and rats as animal model for potency test in addition to dogs. It was found that rat was the most suitable animal for such purpose when inoculated with 1 ml of the vaccine subcutaneously exhibition the highest titer of serum neutralizing antibodies on the 3rd week post vaccination (1.5 $\log_{10}$) and 1.66 antibody titer of ELISA. In Guinea pigs, these antibody titers reached 1.2 $\log_{10}$ and 1.45 by serum neutralization test and ELISA respectively by using a dose of 2 ml of vaccine. These results came in parallel to those obtained by vaccination of puppies. So, rat could be used as an animal model for potency test of canine parvo vaccine instead of dogs.

Introduction

Canine parvo virus (CPV) causes an acute, sometimes fatal gastroenteritis in dogs especially young puppies which succumb to the infection with severe myocarditis leading to sudden death (Appel et. al., 1979 and Carpenter et. al., 1980).

Regarding Egypt, CPV infection has been first reported to occur in police dogs as indicated from clinical and histopathological finding (Bucci et. al., 1982) and seroprevalence by Abd El-Ghany (1988). The most effective means of preventing CPV is the vaccination either by live or inactivated vaccine. Attyat (1994) prepared the first local live attenuated tissue culture vaccine. An inactivated tissue culture CPV was prepared by Attyat et. al., (1998).

Traditionally, a potency test involves the vaccination of the target species followed, after a suitable interval, by its challenge with the virulent organism. This type of test necessitates the death or infection of unvaccinated control animals and those insufficiently protected, and is becoming increasingly less acceptable from both practical and economic viewpoints. So, the aim of this study is the use of animal models other than dogs to test the potency of "CPV" vaccine.

Material and Methods

1- Vaccine:

An inactivated cell culture (CPV) vaccine prepared according to Attyat et al., (1998) was subjected to the present study at Department of Pet Animal Vaccine Research, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

2- Animals:

Four puppies of 3-4 months free from CPV antibodies as screened by serum neutralization test in addition to groups of 40 hamsters (150-

200 gm), 60 adult rats (100-120 gm) and 60 Guinea pigs (250-300 gm) were used as animal models.

3- Experimental Design:

Two puppies were vaccinated subcutaneously (S/C) using a dose of 2 ml/animal containing $10^{6.5}$ $TCID_{50}$/ml of CPV. These animals were boostered two weeks later by the same CPV vaccine. Serum samples were obtained for serum neutralization test (SNT) and ELISA on week interval post vaccination up to 3 months. The other two puppies were left as control.

The route and dose of vaccination in different laboratory animals were tested where hamster, rat and Guinea pig were vaccinated through the intraperitoneal (I/P) and subcutaneously (S/C) using doses of 0.5 ml, 1 ml and 2 ml. These doses were used according to James et al., (2002). Serum samples were collected from vaccinated animals for SNT and ELISA on week intervals.

Ten Guinea pigs and 10 rats were vaccinated (S/C), these animals were boostered after 2 weeks and challenged by the oral route on the 4^{th} week post preliminary vaccination. Another 10 for each Guinea pig and 10 rats were left as animal control and sera sample were collected up to 3 months.

4- Virulent virus:

CPV virulent virus obtained from James Baker Institute, NY, USA with a titer of $10^{3.5}$ $TCID_{50}$/ml on NLFK cells was used to challenge animals by 0.5 ml oral route.

5- Serological techniques:

5.1. Serum neutralization test (SNT):

This technique was applied according to Bass et al., (1982) and the antibody titer was calculated according to Reed and Muench (1938).

5.2. Enzyme Linked Immunosorbent Assay (ELISA):

It was carried out according to Voller et al., (1976). The CPV antigen was prepared using polyethyelen glycol (6000 M.W).

Results and Discussion

It is well known that the antibody response to vaccination in a laboratory animal model can be used as an indirect measure of the immunogenicity of certain vaccines as using mice and Guinea pigs for rabies vaccines (WHO, 1973), cats for AIDS and birds for malaria as mentioned by James et. al., (2002). This approach would seem to appropriate for testing inactivated canine parvo virus vaccine.

In the present study the potency of an inactivated CPV tissue culture vaccine adjuvanted by aluminum hydroxide gel was tested. Different animal species (Guinea pig, hamster, rats and mice) as animal models in addition to dogs as the specific host were used for such purpose.

Data presented in table (1) demonstrated that the best route and dose in vaccinated animal models were in rats and Guinea pigs. In case of rat vaccinated by S/C route in a dose of (1 ml) a titer of 1.5 log_{10} was obtained by SNT on the 3^{rd} week post vaccination (WPV). While in Guinea pigs the SN antibody titer reached to 1.5 log_{10} with

the same route but with double dose (2 ml) on 3rd (WPV). This means that both of rats and Guinea pigs gave (SN) antibody titer higher than hamster which didn't gave (SN) antibody titer not more than 0.9 either by (S/C) or (I/P) routes. These results are similar to those obtained by Senda et al., (1989) who found that rats showed the highest antibody response. Also, it was showed that there was a dose related response in case of rat and Guinea pigs where 0.5 ml gave lower antibody titer than that obtained with 1 ml or 2 ml of the vaccine. These results are confirmed by the results obtained by Goddard and Nicholas (1990) who used chicken as a model for potency test of CPV vaccine and found that administration of the vaccine volumetrically gave higher level of antibodies than administrating the vaccine diluted with saline but the differences were not significant.

Table (2) which showed the results of vaccination of Guinea pigs, rats and dogs by S/C route by a dose of vaccine 2 ml/animal for dog and Guinea pigs while the dose for rat was 1 ml. It was found that the sequence of SN antibody was increased from the 1st week till reached 1.2, 1.5 and 1.8 on 3rd WPV in Guinea pigs, rats and dogs respectively. After oral challenge with the virulent virus of CPV the antibody titers reached to 1.5, 1.9, 2.9 $\log_{10}$ by SNT. These results are confirmed by the results of ELISA as shown in table (3), showing no remarkable difference between Guinea pigs, rats and dogs. Also, from table (2), the rats showed a higher SN antibody titer than Guinea pigs as animal model. These results came in agreement with Senda et al., (1989) who mentioned that rat showed the highest and fastest antibody response in comparison to rabbit, mice and Guinea pigs.

The unvaccinated challenged dogs showed viraemia and diarrhea, while Guinea pigs and rats didn't show abnormal clinical symptoms after challenge. This could be explained according to Siegl (1988) who mentioned that parvo viruses can be divided into viruses easily crossing the species barrier and those replicating and inducing disease in their natural host species. It was known that CPV is serologically and genetically closely related to feline parvovirus (Appel et. al., 1979) and (Siegl, 1988). Feline parvo virus when inoculated into dogs, ferrets, rhesus monkey, hamster, Guinea pigs, mice, rat and chick embryo didn't produce any signs of disease (Lawrence et. al., 1940) and (Siegl, 1988).

Control of species host range among viruses from feline parvo and CP virus group is determined by a similar cluster of surface amino acid changes in the capsid (Truyen and Parrish, 1992). These changes are known to act via species, specific interaction with the viral cell surface receptor recently known as "Transferrin receptor". Canine cell infection is a specific property of CPV and depend on the ability of this virus to bind the canine transferring receptor (Hueffer et. al., 2004).

The results obtained in this study encourage using rat as animal model for potency test of inactivated CPV vaccine where the relative antibody levels produced in rat represent the relative potency achieved in dogs.

Table (1) : CPV neutralizing antibody titers in different vaccinated animal models using different doses and routes of vaccination

Week post vaccination	Mean CPV neutralizing antibody titers (log_{10} ml)																								
	Hamster						Guinea pig						Rat						Mice						dogs
	I/P			S/C			I/P			S/C			I/P			S/C			I/P			S/C			S/C
	Dose			Dose			Dose			Dose			Dose			Dose			Dose			Dose			Dose
	0.5 ml	1 ml	2 ml	0.5 ml	1 ml	2 ml	0.5 ml	1 ml	2 ml	0.5 ml	1 ml	2 ml	0.5 ml	1 ml	2 ml	0.5 ml	1 ml	2 ml	0.25 ml	0.5 ml	1 ml	0.25 ml	0.5 ml	1 ml	2 ml
1st week	0.3	0.3	0.6	0.3	0.3	0.3	0.3	0.3	0.6	0.3	0.3	0.3	0.3	0.3	0.6	0.3	0.6	0.6	0.3	0.3	0.3	0.3	0.3	0.6	0.6
2nd week	0.3	0.3	0.6	0.3	0.6	0.6	0.3	0.6	0.9	0.45	0.9	1.2	0.45	0.9	0.9	0.6	1.2	1.2	0.3	0.3	0.6	0.3	0.6	0.9	0.9
3rd week	0.3	0.3	0.6	0.3	0.6	0.9	0.6	0.9	1.2	0.9	1.2	1.5	0.6	1.2	1.2	0.6	1.5	1.5	0.6	0.6	0.9	0.6	0.6	0.9	1.2

CPV = canine parvo virus.

SNT = serum neutralization test.

I/P = intraperitoneal

S/C = subcutaneous.

Table (2) CPV neutralizing antibody titer in animal vaccinated subcutaneously and intraperitonealy with inactivated CPV

Weeks post vaccination	Guinea pig		Rats		dogs
	S/C	I/P	S/C	I/P	S/C
1st	0.3	0.3	0.35	0.3	0.6
2nd (boostered)	0.6	0.6	0.75	0.6	1.2
3rd	1.2	0.6	1.5	1.2	1.8
Challenged orally 4th	1.5	1.2	1.7	1.2	2.1
6th	1.5	1.2	1.9	1.7	2.3
12th	1.5	1.2	1.9	1.7	2.3

Table (3) CPV ELISA antibody titers in vaccinated animal with inactivated CPV

Weeks post vaccination	Guinea pig		Rats		dogs
	S/C	I/P	S/C	I/P	S/C
1st	0.35	0.3	0.40	0.35	0.68
2nd (boostered)	0.64	0.6	0.87	0.75	1.35
3rd	1.45	1.2	1.66	1.6	1.94
Challenged orally 4th	1.75	1.6	1.9	1.6	2.45
6th	1.78	1.6	1.98	1.9	2.58
12th	1.78	1.6	2.1	1.9	2.62

References

Abd El-Ghany,M. (1988): "Studies on parvo virus in dogs". M.Vet.Sc.Thesis (Microbiology), Fac.Vet.Med., Cairo University.

Appel,M.; Cooper,B.J. and Crmichael,L.E. (1979): "Canine viral enteritis. I-Status report on corona and parvo like enteritis". Cornell Vet., 69: 123-133.

Attyat,M.Kotb (1994): "Studies on the preparation of canine parvo virus vaccine". Ph.D.Vet.Sci.Thesis (Microbiology), Fac.Vet.Med., Cairo University.

Attyat,M.Kotb; Habshi,Y.Z. and Saber,M.S. (1998): "Studies on preparation of an inactivated canine parvovirus vaccine". 4th Vet.Med.Zag.Conf., pp. 240-247.

Bass,E.P.; Gill,M.A. and Becknehauer,W.H. (1982): "Development of a modified live canine origin parvo virus vaccine". J.A.V.M.A., 181(9): 909-913.

British Pharmacopia (Vet.) (1985): London, HMSO.

Bucci,T.J.; Botros,A.M. and El-Malla,M. (1982): "Canine parvo virus infection: A brief review and report of 1st cases in Egypt". J.Egypt.Vet.Med.Assoc., 42 (1): 21-25.

Carpenter,J.L.; Roberts,R.M. and Harpster,N.K. (1980): "Intestinal and cardiopulmonary forms of parvo virus infection in a litter of puppies". J.Am.Vet.Med.Assoc., 179: 1269-1273.

Goddard,R. and Nicholas,P.R. (1990): "Inactivated canine parvo virus vaccine: an alternative method for assessment of potency". Vet.Record, 126: 497-499.

Hueffer,K.; Palermo,L.M. and Parrish,C.R. (2004): "Parvo virus infection of cell by using variants of the feline transferring receptor altering dathrin mediated endocytosis membrane domain localization and capsid binding domains". J.Virol., 78: 5601-5611.

James,G.M.; Lynn,C.Anderson; Franklin,M.L. and Freed Quimby (2002): In "Laboratory animal medicine procedure". Animal model in biomedical research, pp.

Lawrence,J.S.; Syverton,J.T.; Shaw,J.S. and Smith,F.P. (1940): "Infectious feline agranulocytosis". Am.J.Pathol., 16: 333-354.

Reed,L.J. and Muench,H. (1938): "A simple method for estimating fifty percent end points". Am.J.Hyg., 27: 493-497.

Senda,A.H.; Rayama,N.; Ctoh,O.; Yamamoto,H. and Kurata (1989): "A novel potency test for inactivated canine parvo virus vaccine using rats". Annual report of the national veterinary assay laboratory, No. 26: 23-27.

Siegl,G. (1988): "Patterns of parvo viral disease in animals". Pattison,J.R. (ed.) parvoviruses and human disease : Boca Raton, CRC Press, 43-68, chapter 3.

Truyen,U. and Parrish,C.R. (1992): "Canine feline host range of canine parvo virus and feline panleukopenia virus: distinct host cell tropism of each virus in vitro and in vivo". J.Virol., 66: 5399-5408.

Voller,A.; Bidwell,D.E. and Ann Bartlett (1976): "Enzyme immuno-assay in diagnostic medicine: theory and practice". Bull. World Health Organization, Vol. 53: 55-65.

World Health Organization (1973): "Laboratory techniques in rabies". Monograph, 3rd edition, Series No.23, pp. 279-291.

الملخص العربي

إستخدام نماذج حيوانات غير الكلاب لإختبار كفاءة بارفو الكلاب

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**المعمل المركزي للرقابة على المستحضرات الحيوية البيطرية – القاهرة

في هذه الدراسة تم حقن حيوانات مختلفة (الجربوع السوري – خنزير غينيا – الجرذان) بجرعات وطرق مختلفة للحقن تحت الجلد وفي الغشاء البريتوني لإستخدامهم كنموذج لقياس المناعة إلى جانب إستخدام الكلاب وذلك للقاح البارفو النسيجي المثبط. وقد وجد أن الجرذان هي أنسب الحيوانات المحقونة حيث أن ١ سم من اللقاح عندما يحقن تحت الجلد يعطي أعلى معدل من الأجسام المناعية المتعادلة وذلك في الأسبوع الثالث بعد الحقن حيث وصلت إلى (١,٥ لو ١٠) وبإختبار الإليزا وصل إلى ١,٦٦. ووجد أن خنازير غينيا وصل معدل الأجسام المناعية إلى ١,٢ لو ١٠ و ١,٤٥ بإستخدام السيرم المتعادل وإختبار الإليزا على التوالي ولكن بإستخدام جرعة ٢ سم من اللقاح. كانت هذه النتائج موازية تماماً لما تم الحصول عليه عن حقن الكلاب ولذلك نستطيع أن نستخدم الجرذان كحيوان نموذج لقياس المناعة للقاح البارفو المثبط.