Efficacy of canine parvovirus hyperimmune serum prepared in horses for treatment of canine parvo and feline panleucopenia infections

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ABSTRACT

Anti-canine parvovirus hyper immune serum was successfully prepared in horses where it was found to have specific canine parvovirus (CPV) neutralizing antibodies of a titer 1024/ml as determined by serum neutralization test (SNT). Quality control testing of such serum revealed that it is free from bacterial, fungal and mycoplasma contaminants as tested on specific media and safe as tested in puppies. Passive immunization of CPV naturally and experimentally infected dogs and feline panleucopenia (FPL) naturally infected cats with the prepared antiserum through inoculation of 2ml/ animal I/M for 5 successive days prevent the disease progress and mortality when administrated 2 days before infection and up to 3 days post experimental infection. The protection decreased to 66% with delayed treatment on the 4th day and become non protective at the 5th day post experimental infection. So it could be concluded that specific anti-CPV hyper immune serum could aid to treat or even decrease the dangers of CPV and FPL infection among dogs and cats when administered early to infected animals.

Keywords: canine parvo virus, feline panleucopenia, hyperimmune serum

(http://www.bvmj.bu.edu.eg) ***conference issue*** **(BVMJ-28(2): 34-39, 2015)**

1. INTRODUCTION

Canine Parvovirus (CPV) infection is one of the most common infectious diseases of dogs and the most prevalent viral cause of diarrhea in dogs. It is postulated to have arisen either from feline pan leucopenia or a wild carnivora parvovirus. Most affected dogs are under 6 months of age. Ten to twelve weeks of age is particularly vulnerable due to the waning protective effect of maternally derived antibodies or due to failure of vaccination (Greene and Decaro, 2012). Sudden onset of hemorrhagic diarrhea; fever and leucopenia in young unvaccinated dogs is often considered indicative of CPV infection. However, not all dogs with CPV have bloody diarrhea or leucopenia and other parasitic or entero-pathogenic bacterial infections can cause such symptoms. The definitive diagnosis of these causative agents should be pursed (Karen, 2014). Preventing disease after exposure to a biological agent is partially a function of the immunity of the exposed individual. Unlike vaccines, which require time to induce protective immunity, it depends on the host's ability to mount an immune response (Casadevall, 2002). Passive antibodies can theoretically confer protection regardless of the immune status of the host. Passive antibody therapy has substantial advantage over antimicrobial agent and other measures for post exposure prophylaxis including high specific antibody as rabies (WHO, 1996 and Khodier and Daoud, 2008) and canine parvo (Hoskins, 1998 and Nguyen et. al, 2006). The present work was designed to prepare horse anti-CPV hyperimmune serum with evaluation of its efficacy to be used in emergency cases of CPV infection in dogs and feline panleucopenia in cats.

2. MATERIAL AND METHODS

2.1. Inactivated Canine parvo virus vaccine:

An inactivated cell culture canine parvo vaccine (Attyat et al., 1998) was supplied by the Department of Pet Animal Vaccine Research (DPAVR), Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo. It was used for preparation of CPV hyperimmune serum in horses.

2.2. 2- Canine parvo virus (CPV):

Cell culture adapted CPV: BHK-21 cell culture adapted strain of CPV with a titer of 10^7 $TCID_{50}$ /ml (Attyat, 1994) was supplied by DPAVR and used for estimation of CPV antibodies in the prepared antiserum using SNT. *Virulent CPV strain:* Virulent CPV strain adapted on Norden Laboratory feline kidney (NLFK) cell culture with a titer of $10^{3.5}$ $TCID_{50}$ /ml (Attyat, 1994) was used for experimental infection of puppies through the oronasal route.

2.3. Animals:

2.3.1. Horses:

Three native breed horses of about 1.5-2 years old were used for preparation of CPV hyper immune serum. These horses were found to be healthy and free from external and internal parasites.

2.3.2. Dogs:

Three naturally CPV infected dogs; 6-9 months old suffering from vomiting; pyrexia and bloody diarrhea were delivered by the public veterinary hospital. Their fecal swabs reacted positive with CPV antigen detection kit. These dogs inoculated I/M with 2 ml/dog of the prepared horse anti-CPV serum for 5 successive days. Thirty native breed puppies of 3-4 months age free from internal and external parasites and free from CPV antibodies, were divided into 10 groups (3 puppies/ group) as follow: Group1: Received horse anti-CPV antiserum 2 days before experimental infection with CPV. Group2: Received horse anti-CPV antiserum 1 day before experimental infection with CPV. Group3: Received horse anti-CPV antiserum on the day of experimental infection with CPV. Group 4 to group 8 were experimentally infected and received horse anti-CPV antiserum on 1, 2, 3, 4 and 5 days post infection, respectively. Group 9: was experimentally infected and kept without treatment. Group 10: Received horse anti-CPV antiserum; 2ml/dog for 5 successive days. Virus infection was carried out through the oronasal route, while the antiserum was inoculated I/M using a dose of 2 ml.

2.3.3. Cats:

Two Shiraz's cats suffering from clinical signs of FPL delivered by the same hospital and their fecal swabs reacted positive with CPV antigen detection kit, were inoculated I/M with horse anti-CPV serum (2ml/cat) for 5 successive days. Dogs and cats were subjected to daily clinical examination for recording of clinical signs of CPV and FPL and deaths. Serum samples were collected from the survivals every week up to the 3rd week post treatment to monitor CPV antibody titers.

2.4.CPV- antigen detection kit:

CPV antigen detection rapid test kit supplied by Bio Note Koren (445-170) was used for detection of CPV and feline panleucopenia (FPL) in naturally infected dogs and cats in fecal swabs.

2.5.Preparation of anti-CPV hyper immune serum:

Preparation of anti-CPV hyper immune serum was carried out in horses using increasing doses of the inactivated CPV vaccine; 10, 20, 30 and 40 ml on a week interval injected through 2 different routs (S/C and I/M) according to the method described by (Atanasin and Lepine, 1973). Serum was collected 14 days after the last injection. The prepared antiserum was subjected to sterility tests (aerobic and anaerobic bacteria, fungus and mycoplasma contamination) following the directions of WHO (1992).

2.6.Serum neutralization test (SNT):

SNT was carried out using the microtiter technique according to Bass et al., (1982) to estimate the CPV-neutralizing antibody titers in sera of immunized horses, dogs and cats. The antibody titer was calculated as the reciprocal of the final serum dilution, which neutralized and inhibited the CPE of 100$TCID_{50}$ of CPV according to Singh et al. (1967).

3. RESULTS

Preparation of anti-CPV hyper immune serum: Following up the level of anti-CPV hyperimmune serum weekly in horses revealed a gradual increase in titer after the 1st week from 16 units up to 1024 unit 2 weeks after the last injection (table 1). The prepared serum was proved sterile and save in puppies (table 2). Efficacy of anti-CPV hyperimmune in the protection and treatment of experimentally infected dogs: It was observed that administration of the anti-CPV hyperimmune serum for dogs 2 and 1 day before experimental infection and on the day of experimental infection prevent the appearance of clinical signs of canine parvo infection. In addition, administration of the anti-CPV after 1, 2 and 3 days post experimental infection prevent progression of the disease and relief of the already present symptoms. On group 7 one of the dogs died due to the delayed injection of anti-CPV. Mean titer of CPV serum neutralizing antibodies in infected, treated and survived animals: the antibody titer declined rapidly in animals one week post last injection and continue decreasing till the 3rd week. It diminished completely at the 3rd week in group 7 of experimentally infected dogs and in naturally infected cats.

Table (1): Following up the level of anti-CPV serum neutralizing antibodies in immunized horses

Titer of anti-CPV serum neutralizing antibodies/WPI*				
1WPI	2WPI	3WPI	4 WPI	5 WPI
**16	64	256	512	1024

*WPI= week post immunization. **Titer of CPV antibodies= the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 $TCID_{50}$ of CPV.

Table (2): Quality control parameters of the prepared anti-CPV hyper immune serum

Sterility	CPV serum neutralizing antibody titer	Safety in puppies
Free from aerobic and anaerobic bacteria; fungi and mycoplasma	1024/ml	No significant local or systemic signs

4. DISCUSSION

Improper vaccination protocol and vaccination failure may lead to CPV infection. In addition, breeding kennels and dog shelters that hold a large number of inadequately vaccinated puppies are particularly hazardous places. Also puppies become susceptible to virus infection in contaminated environment (Karen, 2014). Treatment of CPV infection can be quite costly. So one of the treatment protocols is the administration of homologous antiserum from immune dogs (Meunier et al. 1985). Hyper immune sera contain antibodies which are natural product with minimal toxicity, provide that they have no reactivity with host tissue (Tizard and Ni 1998). Serum neutralization test indicated that the prepared antiserum had a titer of 1024 (table-1), which appears to be

Table (3): Efficacy of anti-CPV hyper immune serum in the treatment of experimentally infected dogs

	2DBI* Group1	1DBI Group 2	DI** Group3	1DPI*** Group4	2DPI Group5	3DPI Group6	4DPI Group7	5DPI Group 8	Grou p9	Group 10
Number of treated dogs	3	3	3	3	3	3	3	3	3	3
Number of survived dogs	3	3	3	3	3	3	2	0	0	3
Protection %	100	100	100	100	100	100	66	0	0	100
Recorded clinical signs	NCS#	NCS	NCS	Lethargy , loss of appetite	Fever, vomiting, diarrhea	Fever, vomiting, diarrhea	Bloody diarrhea, vomiting, fever,dep -ression	Death	Death	NCS

*DBI= day before infection. DI= day of infection. ***DPI= day post infection. #NCS= no clinical signs

Table (4): Mean titer of CPV serum neutralizing antibodies in treated animals

Treated animals		Titer of anti-CPV serum neutralizing antibodies / WPT* 1WPT	2WPT	3WPT
Naturally infected dogs		**64	32	8
Experimentally	Group 4	64	32	16
infected, treated	Group 5	32	16	4
and survived	Group 6	32	8	2
dogs	Group 7	16	8	0
Naturally infected cats		16	4	0

*WPT= week post treatment. **Titer of CPV antibodies= the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 $TCID_{50}$ of CPV

supported by what reported by Atanasin and Lepin (1973) and Macartney et al. (1988). Such antibody titer was noticed to increase gradually from the 1st to the 5th week of horse immunization (2weeks post last injection), confirmed by the findings of Pollock and Carmichael (1982). The obtained results revealed that the prepared horse anti-CPV hyper immune serum was free from foreign contaminants (aerobic and anaerobic bacteria; fungi and mycoplasma) and safe when inoculated in puppies (group 10 in table 3) showing no significant local or systemic reactions or deaths (table-2). These findings come in agreement with the recommendations of WHO (1996). On the other hand, naturally and experimentally infected dogs did not show local reactions at the site of injection or significant systemic reactions with the prepared anti-CPV serum; confirming its safety for dogs. The same safety observations were reported in treated naturally infected cats with FPL. However, these animals exhibited good levels of passive antibody titers up to 3 weeks post last injection of anti-CPV hyperimmune serum as shown in table (4) then declined gradually. In addition treated animals showed health improvement within five days post treatment with the antiserum, except one out of 3 treated naturally infected dogs, which died. It could be attributed to its late stage of infection or due to the incidence of other factor as bacterial toxins which is common in case of enteritis as reported by WHO (1992). In case of experimental infection of puppies with the virulent CPV; it was found that administration of CPV antiserum resulted in

100% protection when it was administrated on the 1st and 2nd day before virus infection and on the day of infection and up to 3 days post infection while the protection percentage was 66% when the antiserum administered on the 4th day and become non protective at the 5th day post experimental infection (table-3). The 9th group; receiving virus infection only died by the 5th day post experimental infection showing bloody diarrhea and fever. From the above result, it could be observed that passive immunization against CPV and FPL infections with specific antibodies could be used during the first days of infection to have adequate efficacy. These findings appear to be similar to those obtained by Pollock and Carmichael (1982) and Meunier et al. (1985) who used dog immune serum to protect dogs from experimental oral infection. Also frozen plasma is used now for treatment of CPV and FPLV infections (Casadevall, 2002) in addition to the use of egg yolk, which is applicable now for the same purpose (Greene, 2006). We can suggest that passive immunization of puppies before experimental infection with CPV resemble the maternal immunity, which could overcome virus infection. Therefore, we could conclude that the use of CPV hyper immune serum had been beneficial in the treatment of naturally infected dogs with CPV and naturally infected cats with FPLV as it minimize the disease severity in addition to the reduction in treatment and hospitalization time and cost. Further studies are in need to evaluate the use of different doses and different routes for administration of CPV antiserum.

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