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Efficacy of Canine Distemper and Canine Parvo Antisera to Overcome Viral Infections in Dogs

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Abstract: ***The authors have been succeeded through this work to prepare horse anti-canine distemper (CD) and anti- canine parvo (CP) hyper immune serum as a local product provided on time of request instead of the imported one which is usually unavailable and of high cost. Such antiserum was found to be free from foreign contaminants and safe inducing no abnormal local or systemic signs in inoculated animals. It was found that this antiserum had CD and CP antibody titers 512 and 1024 respectively as titrated by the Antibody Quantitate ELISA kits. These levels of antibodies were able to overcome CD in naturally infected and contact infected puppies in a percentage of 33.3 and 100% respectively. While in case of CP naturally infected and in contact infected puppies this ratio was 100% so it could be said that the prepared horse anti-CD and anti-CP hyper immune serum could be used as emergency management of CD and CP diseased puppies lowering disease progress and economic losses caused by death of these animals.This work indicated also that the Chromatographic Immunoassay as a rapid assay for detection of canine distemper (CD) and canine parvo (CP) virus antigen was less sensitive than other serological tests giving positive results of 57.1 and 54.4% of examined samples to CD and CP antigens respectively confirming the recorded disease signs on naturally infected puppies.***

Keywords: canine distemper, canine parvo, hyper immune serum

1. Introduction

Canine distemper virus (CDV) is classified as a member of the family *Paramyxoviridae*, genus *morbilliviruse*, which includes phocine distemper, measles, pest des petits ruminants and rinderpest viruses. Canine distemper virus has envelope and a negative-sense Single-stranded genome (negative-sense RNA). It is the etiological agent of a severe and highly contagious disease which has a high mortality rate in susceptible dogs and many other carnivores, that yield multi-systemic disease that can be present with one or more of the following forms, respiratory disease with severe pneumonia, gastrointestinal disease with vomiting and diarrhea, neurological disease including seizures and severe immunosuppression leading to infection by normally harmless bacteria and viruses [(1,2)].Canine distemper virus (CDV) is a contagious viral pathogen causing death in both domestic and wild dogs [(3)]. Although immunization against canine distemper has been applied over beamy scales for many years ago, this infection still represents a significant disease for many other carnivores and dogs. By immunization we can make control the infection of dogs with Canine distemper virus (CDV), but the vaccine types in present use are linked with a number of the problems happened such as confusion with maternal antibodies so that sometimes the immunized dogs become liable to infection after immunization [(4)]. The value of the humeral immune reaction in these states is indicated by the truth that the prime infection of dogs by CDV often leads to lethal problems, when the dog does not yield the efficient effective early neutralizing antibody response [(5)].Canine parvovirus (CPV) was identified in the 1970's that has regularly transformed into new virulent forms [(6)]. CPV is non-enveloped and single stranded DNA virus belonging to the family of *Parvoviridae*. There are now three widely documented strains of canine parvovirus: CPV-2A, CPV-2B and CPV-2C[(7, 8)],CPV presented as one form, CPV2, when discovered. With time, the virus has mutated into multiple subsets: CPV-2A, CPV-2B, and CPV-2C[(9)]. The modern form, identified in 2000 in Italy, is CPV-2C and is the most common illness seen in the U S. Intensity of the illness depends upon two factors; viral quantity and viral type [(10)]. CPV2 is one of the most common enteric pathogens seen in canines and predominantly infects neonates of different species [(6)]. CPV infects dogs and induces severe gastrointestinal and cardiac signs where the diarrhea occurs in dogs of any age but appears in serious proportions in pups. Early symptoms are depression, loss of appetite, vomiting, high fever and severe diarrhea .There is slight rise of temperature in the initial stage of the disease but gradually turn to subnormal level with advancement of vomiting and diarrhea [(11)].The faeces of infected dogs considered as source of infection to other susceptible dogs [(12,13)]. CPV infection has two forms of clinical symptoms, the 1st form characterized by enteritis with vomiting and bloody diarrhea in dogs of all ages [(14,15,13)] while the 2nd form characterized by myocarditis and heart failure in pups of less than 3 months of age [(16)]. Canine parvovirus is extremely

infectious disease and is highly spread from dog to another throw the direct or indirect contact with their faeces. The vaccines have been the most successful biomedical invention to prevent the morbidity and mortality of humans and animals caused by infectious diseases [17, 18].Newly born animals are partially protected against prevalent infections by maternally transferred immune effector mechanisms, most especially maternal antibodies [19,20]. From time to time the vaccinated dogs become susceptible to infection after vaccination, that's due to number of the problems like as the interfering of maternal antibodies so that sometimes the vaccinated pups become susceptible to infection after vaccination [4]. The procedures by which the immune response promotes recovery from viral infection, including lysis of infected cells, viruses' lysis, and viruses' neutralization [21]. Hyper immune sera have antibodies which are natural product with negligible toxicity, provide that they have no reaction with host tissues [22]. Depending on such fact, it seems that there is a need for the development of highly potent preparations such as specific hyper immune serum, which are conventionally used in emergency cases and this is the goal of the present work aiming to provide CD and CP hyper immune serum to be available on time of need as local products saving time and cost.

2. Materials and Methods

1) Animals

a) Horses

Two apparently healthy native breed horses, free from external and internal parasites of about 4 years old were housed under hygienic measures receiving balanced ration and adequate clean water were used for preparation of hyper immune serum against canine distemper and canine parvo viruses.

b) Puppies

Healthy puppies

Nine apparently healthy native breed puppies were divided into three groups; three of them were kept in contact with puppies infected with canine distemper virus (1st group), three dogs were kept in contact with puppies infected with canine parvo virus (2nd group) and three dogs were kept as negative control (3rd group) .

Diseased puppies

Seven puppies were apparently infected with canine distemper and other 11 puppies were apparently infected with canine parvovirus delivered to the veterinary hospital were subjected for treatment trials with the prepared mixture of CD and CP hyper immune sera. CD infected puppies were suffered from fever, nasal discharge and diarrhea while CP infected puppies were suffered from general weakness, vomiting and bloody diarrhea.

Mice

Fifteen weaned Swiss Albino mice supplied by Veterinary Serum and Vaccine Research Institute (VSVRI) were used to test the safety of the prepared anti-CD and CP horse sera.

2) NobivacR Puppy DP vaccine (canine distemper and canine parvo vaccines)

CD and CP bivalent live attenuated vaccine with a titer of 10^7 TCID50 /ml was supplied by Intervet International, Holland and used for preparation of CD and CP hyper immune sera in horses.

3) Detection of virulent CD and CP viruses

Virulent CD and CP viruses were detected in nasal swabs and fecal swabs respectively from the naturally diseased puppies through application of the Chromatographic Immunoassay.Bionote Antigen Rapid CD and CPV Ag test kits were manufactured and supplied by Bionote Korean Company.

4) Immune-stimulant drug

Zylexis injection which is Parapox ovis virus immunomodulator Mfd. By: Zoetis Belgium SA and used as nonspecific immune stimulant for horses used for preparation of anti-CD and CP immune sera.

5) Preparation of anti-canine distemper and anti- canine parvo hyper immune sera

Preparation of CD and CP hyper immune sera was carried out in horses using a dose of CD and CP bivalent live attenuated vaccine 1ml/ subcutaneously for five successive doses according to the method described by Atanasin and Lepine [23].Zylexis was supplied by Zoetis containing inactivated *P. ovis* strain D 1701 (with a minimum of 230 IFN units) was used on days 0, 2, and 4. Dose frequency and route of admission were chosen according to the manufacturer's recommendations. The used dose was 2ml/horse inoculated subcutaneously. Serum samples were obtained from inoculated horses on week intervals post inoculation then serum was collected one week post the last injection and subjected for titration of induced canine distemper and canine parvo antibodies through application of Antibody Quantitate ELISA. The prepared hyper immune sera were subjected to sterility tests and proved to be free from aerobic and anaerobic bacteria, fungi, and mycoplasma contaminations following the directions of WHO[24].

6) Lyophilization of the prepared anti-canine distemper and anti- canine parvo hyper immune sera

The prepared hyper immune sera were stabilized with lactalbumin and sucrose in a ratio of 1:1 (w/w). The mixture solution (4ml) was dispersed in neutral glass vials under sterile conditions and lyophilized following the method adopted by Sisti et al[25], where the prepared mixture was frozen and held at –40°C for 6 hours at atmospheric pressure and subsequently the temperature was decreased to –50°C for another 10 hours (super freezing), at a vacuum of 10 m par and maintained during the rest of the process. The primary drying time was 48 hours, after which the temperature was gradually increased to 35°C and maintained for a further 8–10 hours. The lyophilized antiserum was stored at -20°C.

7) Titration of CD and CP antibodies in the prepared horse antiserum

The titer of CD and CP antibodies in the prepared horse antiserum was determined using CD and CP virus Antibody Quantitate ELISA kits supplied by Shenzhen Zhenrui

Biotech Co., Ltd. The antibody titers were calculated according to the instruction directions.

8) Experimental infection of healthy puppies

Three CD diseased puppies were housed with three healthy puppies and three CP diseased puppies were housed with three healthy puppies. Each animal group was housed separately from the other group.

9) Trials of treatment of naturally diseased and experimentally infected puppies

Naturally CD and CP diseased puppies and contact infected puppies were treated with different doses of hyper immune serum against the two diseases (0.5, 1, 2, 3ml) it was found that 2ml dose was the dose of choice giving the best protection results so that the prepared CD and CP hyper immune serum was injected in a dose of 2ml by I/V rout for five successive days according to Attyat et al [26].

3. Results and Discussion

The recorded disease signs on naturally suspected CD infected puppies included fever, nasal discharge and diarrhea. These signs agree with those reported by Apple [27], Kapil and Yeary [1] and Espinal et al (2). On the other side naturally CP infected dogs showed vomiting, bloody diarrhea and death of 6 out of 11 affected dogs. Similar findings were recorded by Appel et al [14]; Woods et al [15]; Nandi and Kumar [11] and Tilley and smith [10].

Table 1: Detection of CD and CP antigens using the Chromatographic Immunoassay

Chromatographic Immunoassay					
Detection of CD antigen			Detection of CP antigen		
Number of tested samples	Number of positive samples	% of positive samples	Number of tested samples	Number of positive samples	% of positive samples
7	4	57.1	11	6	54.4

Application of Chromatographic Immunoassay on nasal swabs from CD infected dogs revealed that 4 out of 7 samples (57.1%) showed positive results indicating the presence of CD virus antigen while 6 out of 11 (54.4%) fecal samples from CPV infected dogs showed positive results of CPV antigen (table-1). In this respect Desario et al[28],Attyat and Wafaa [26] and Rofida [29]stated that Chromatographic Immunoassay (CI) was compared to molecular techniques, showing that the relative sensitivity of the test did not exceed 50% with respect to the nucleic acid-based methods, whereas the specificity was 100%. The poor sensitivity of the CI test was associated with the low amounts of virus shed in the feces during the late stages of infection and/or the early presence of high CPV antibody titers in the gut lumen that may sequestrate most viral particles.

The prepared horse anti-CDV and CPV hyper immune sera were found to be free from foreign contaminants (aerobic and anaerobic bacteria, fungi and mycoplasma) and safe when inoculated in mice showing no local or systemic reactions or deaths (table-2) coming in agreement with the recommendations of WHO[30].

Following up the levels of CD and CP antibodies in the prepared horse antiserum through application of ELISA using Antibody Quantitate ELISA kits, it was found that both antibody levels were increased gradually by the first week post horse immunization (8 for CD and 16 for CP) to reach their peaks (512 for CD and 1024 for CP) one week post the last fifth inoculation (Table-2). These observations came to be supported by what reported by Atanasin and Lepin [23] and Macartney et al [31] and confirmed by those of Pollock and Carmichael [32], Attyat and Wafaa [26] who obtained similar results during preparation of such antisera.

Table 2: Quality parameters of the prepared horse anti-CD and CP serum

Quality parameters of the prepared horse antiserum			
Sterility	Safety in mice	CD-ELISA titer	CP-ELISA titer
Free from foreign contaminants	Showing no local or systemic reactions or deaths	512	1024

Regarding housing of the three healthy susceptible puppies with naturally infected ones with CD, it was noticed that the major symptoms include high fever (39.7° C) red eyes, and a watery discharge from the nose and eyes were observed on the contact pups. Infected dogs became lethargic and tired, and anorexic. These findings appear to be supported and confirmed with what recorded by Pet Med [33].

On the other hand 2 out of three housed healthy puppies with CP infected puppies attracted the infection within 3 days after housing with diseased dogs. The recorded disease signs were characterized by fever, vomiting and bloody diarrhea. These signs came in agreement with those recorded by Saho et al[34] who stated that bloody diarrhea was one of the most clearly observed signs in 40% of CPV infected dogs.

Table 3: Efficacy of the prepared horse anti-CD and CP sera

Treated puppies	Efficacy of the prepared horse serum against					
	CD			CP		
	No. of treated	No. of survived	Protection %	No. of treated	No. of survived	Protection %
Naturally infected	3	1	33.3	3	3	100
In contact infected	3	3	100	3	3	100

Table (3) demonstrated that three of the naturally infected puppies with CD virus disease and the three puppies which were in contact infected received the prepared horse anti-CD and CP serum for 5 successive days where two naturally infected and treated puppies died receiving one dose of the antiserum (33.3% protection) the thing which could be

attributed to the late treatment where they became dehydrated which may be as a consequence to large quantity of fluid loss due to vomiting and diarrhea in agreement with Greene and Decaro [(36)] at the same time all the experimentally infected puppies were able to withstand the infection (100 % protection). While the other three puppies of the naturally infected with CP virus disease and the three puppies in contact infected received the prepared horse anti-CD and CP serum for 5 successive days were able to withstand the infection (100% protection). Similar results were obtained by Attyat and Wafaa [(26)] who recorded that treated dogs showed health improvement within five days post treatment with the CP antiserum, except one out of three treated naturally infected dogs, which died this was 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 [(24)]. It was observed that passive immunization against CPV infections with specific antibodies could be used during the first days of infection to have adequate efficacy as demonstrated by Pollock and Carmichael [(32)] and Meunier et al[(37)] who used dog immune serum to protect dogs from experimental oral infection. In addition Bergman et al [(38)] stated that antibodies play an important role in the defense against infectious diseases. Passive immunization provides immediate protection through transfer of exogenous antibodies to the recipient.

Depending on the present obtained results it could be concluded that the prepared bivalent anti-CD and anti-CP hyper immune serum in horses is -of a valuable benefit and sufficient to overcome both viral infections especially when administered with early appearance of the disease signs and in combination with the other regular treatment regimens used for treating diseased dogs saving animal life and economic losses.

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