INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2006/08–6–898–900
http://www.fspublishers.org

Short Communication

Isolation and Characterization of Canineparvo Virus

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ABSTRACT

A study was conducted to isolate and characterize canine parvovirus. Parvovirus were mainly isolated from 300 faecal samples of parvo-suspected pet dogs and intestinal scrapings/tissues of dogs died of this disease. The presence of the virus in the samples was confirmed and quantified by using haemagglutination (HA) test. Cases were recorded and categorized on the basis of age, breed, season and sex. More cases (47%) were seen in 0 - 2 month age group, German shepherd (Alsatian) breed was more prone (84%), maximum number of cases (61%) was recorded in hot- humid season during the months of July to September and male dogs were more at risk (85%) than female population. Serological characterization of the field virus was done by haemagglutination inhibition (HI) test using anti-CPV antibodies raised in rabbits. Moreover, effect of various physico-chemical factors like storage time (0, 15, 30 & 45 days) and temperatures (25^{o}C, + 4^{o}C, - 10^{o}C & -196^{o}C), pH (3 - 9) and chemicals (antibiotics streptomycin, penicillin, gentamycin & nystatin; sodium azide & Formaldehyde) on the HA potential of the virus using erythrocytes of various species of animals (chicken, rabbit, dog, cat & monkey) was also evaluated. The study elucidated that the titer of virus in the faeces after the onset of the disease might be helpful in prognosis of recovery or fatality of the infected dog. Chicken erythrocytes proved to be a good source of erythrocytes in HA and HI tests. Moreover, chicken erythrocytes showed early agglutination (within 25 min) than erythrocytes of other animals (Rabbit, monkey, dog & cat), which took more time (1 h) to exhibit agglutination.

Key Words: Canine parvoviru (CPV); Hemaggultination (HA); Hemaggultination inhibition (HI); Erythrocytes (RBC); Faecal

INTRODUCTION

The canine parvovirus is an acute viral disease of dogs characterized by onset of vomition and diarrhoea especially in pups (Pollock & Carmichael, 1983). As a result, diseased animals confront a major loss in their body weight. The disease is usually prevalent in unvaccinated dogs due to non-awareness of the owners, high costs of vaccines, poor husbandry, and faulty biosecurity practices. The continuous presence of the pathogens at those premises, therefore, makes the disease endemic for a particular area. Moreover, most of the kennel owners do not bother about the immune status of the bitches before breeding them so the pups, from these mothers usually lack maternal immunity. Poor husbandry for pets especially dogs and cats was associated negative consequences for public health (William *et al.*, 2002).

This study was aimed at isolation and characterization of parvo-virus from dogs clinically suspected for the disease.

MATERIALS AND METHODS

Three hundred clinically suspected dogs (unvaccinated) were examined, which were brought to the Dog Hospital (Pet Center), University of Veterinary and Animal Sciences (UVAS), Lahore and to the various Kennels and private clinics in Lahore area. The cases were recorded with clinical history and symptoms like anorexia, emaciation, vomition, foul-smelled diarrhea (mostly bloody in nature), temperature, depression, rough coat, and color of mucous membranes. Cases were also recorded on the bases of age, breed, season and sex. Faecal samples were collected from the suspected dogs showing classical sign of parvo disease intestine scraping and tissues were collected from the dogs, which were due to parvo disease.

Processing of faecal samples. Faecal samples were processed by mixing equal amount of faecal sample and solution having pH 7.2 and centrifuged at 500 xg for 10 min at 10°C. Supernatant was collected and stored (4°C).

Processing of scrapping. The scrapings collected from mucosal surfaces of the intestine were mixed with the saline solution in ratio of 1:2 weight/volume. Freeze thawing of the sample was done thrice to break the epithelial cells for liberation of virus particles. The material was centrifuged at 500 xg for 10 min at 10°C. The supernatant was collected in sterile universal bottles (25 mL capacity) as virus suspension.

Processing intestinal tissues. The whole intestinal tissue (10 g) was added in 20 mL of the saline solution pH 7.4.

The tissue in the saline solution was homogenized with 10 g of washed and sterilized sand using pestle and mortar. The homogenized material was filtered through fine sterilized muslin cloth to remove the sand. The filtrate was centrifuged at 500 xg at 10°C for 10 min. The supernatant was stored at 4°C for further processing.

Characterization of the Causative Agent

Haemagglutination test. Each stored sample was subjected to haemagglutination (HA) test as described by Fenner *et al.* (1993) using rhesus monkey erythrocytes. Blood was collected from various animals including chicken, rabbit, dog and cat in order to check the haemagglutinating potential of the causative agent to the 1% washed erythrocytes of various animal species.

Serological rising hyper immune sera. Hyper immune serum against the canine parvo virus was raised in rabbits using commercial vaccine (Primadog; Merial, France). Haemagglutination agents were processed for haemagglutination Inhibition test using known serum against vaccinal strain of canine parvovirus for its seroconfirmation (Celer, 1984).

In ovo propagation of agent was done by using 9 days old embryonated chicken and duck eggs. The effect of physico-chemical factors such as pH, storage temperature and chemicals (antibiotics, sodium azide & formaldehyde) were evaluated.

RESULTS AND DISCUSSION

The disease was more common in pups ranging from 1 - 5 months of age. Lack of maternal immunity and poor immuno-competency for the acquired immunity at this age could be incriminated to be the cause of high incidence. Similar observations have been made by Quinn *et al.* (2002), who recorded the disease in dogs between weaning and six month of age. The findings also support Mohan *et al*. (1994), who described that viral replication, had been dependent on the mitotic activity of myocardial and intestinal cells at this stage of development, when weaning took place. Clinical signs of the disease were onset of vomition, diarrhea, foul smell in faeces, dehydration, sunken eyes, dullness and lethargy. Un-clotted blood was mostly observed in the faeces. The death occurred within 3 days in un-treated cases. Similar observations have been recorded by Pollock and Carmichael (1983).

Canine parvo was more commonly observed in dog breeds like German shepherd (84%), in Rottweiller (9%), in Doberman (5%) and in Bull terriers (2%). The disease was not recorded in dogs of non-descriptive breeds. These results are comparable to Glickman *et al.* (1985), who recorded the highest incidence of the disease in Rottweiller and Doberman. In Pakistan, people mostly keep German shepherd dogs as pet animal and this could be the major possible reason for high incidence of the disease in this particular breed.

Incidence of the disease was high between July to September (Table I) and was less prevelant during rest of the year. Similar findings have been reported by Houston *et al*. (1996), who recorded three times higher number of cases of Parvo disease during July to September as compared to rest of the year.

Table I. Percentage of risk factor

Distribution	Condition	Number (%age)
	0-2 Month	141 (47)
Age wise	3-6 Month	123 (41)
	7-9 Month	24 (8)
	10-above Month	12 (4)
	German Shepherd	252 (84)
Breed wise	Rottweilers	27 (9)
	Doberman	15 (5)
	Bull Terriers	6 (2)
	January – March (Spring)	9 (3)
Season wise	April – June (Hot)	87 (29)
	July – September (Hot-Humid)	183 (61)
	October – December (Cold)	21 (7)
Sex wise	Male	255 (85)
	Female	45 (15)

The prevalence was higher in males compared with females. This could be due to the the fact that males usually travel more as vagabond and have more agility than female so they are more prone to field challenge present in the form of faeces on roadsides and parks defecated by parvo-infected dogs. It is generally observed that intact males are more likely to develop enteritis than intact females, reflecting the tendency of male dogs to roam (Anonymous, 1998).

The canine parvovirus was excreted in the highest amount in the faecal material. The virus can be detected in the faecal material by using different techniques including direct microscopy, haemagglutination (HA) test and polymerase chain reaction (Ransburg *et al.*, 1979; Eugster, 1980; Pollock & Carmichael, 1983; Yasuhara *et al.*, 1989; Pereira *et al*., 2000). Similarly, HA test was performed for the isolation of canine parvovirus from the infected faeces by using avian and mammalian erythrocytes. These results are in line with Appel *et al.* (1979), who used pig and rhesus monkey erythrocytes for the test. The HA test is also used for isolation and diagnosis of canine parvovirus by Carmichael *et al.* (1980), Eugster (1980), Studdert *et al.* (1983) and Cavalli *et al.* (2001). The field isolate gave positive haemagglutination inhibition test with known positive serum (Eugster, 1980; Celer, 1984; Cavalli *et al.*, 2001). This result may be helpful in confirming that the haemagglutination activity shown by the isolated virus was due to canine parvovirus. These results also indicate that there was a cross protection between vaccinal strain and field isolate.

Presence of intra nuclear inclusion bodies in gastrointestinal mucosa suggests that these are predilection site of virus multiplication. This could be a possible reason of presence of high HA titer of the virus in faeces, intestinal tissue and scrapings. The highest titer of virus was found in

the faecal material rather than in the scraping and intestinal mucosa of the same dog. These results corroborate with the statement of Fenner *et al.* (1993) that faecal material from dogs of acute condition had 20,000 HA units of virus or 10^9 virus particles per gram of the faeces.

Canine parvovirus agglutinated the erythrocytes of all animal species (rabbit, dog, Cat & Rhesus monkey). Similar observations were made by Celer (1984), Durham and Johnson (1985) and Senda *et al.* (1986). However, it was observed first time that the virus is capable to agglutinate chicken erythrocytes. These erythrocytes showed results of HA within 25 min, while mammalian erythrocytes took one hour to develop results. This could be due the reason that avian erythrocytes are nucleated, heavier and have more density as compared to erythrocytes of other sources. The haemagglutination could be due to presence of haemagglutinin molecules on the virus surface. These molecules being glycoprotein in nature have the ability to bind on the surface of avian and mammalian erythrocytes. The chicken erythrocytes are preferred to use in clinical diagnosis of canine parvovirus disease in sero-diagnostic laboratories as these erythrocytes display result with in half an hour.

The canine parvovirus isolated from faecal samples when inoculated in chicken or duck embryos failed to grow. Feline pan-leukopenia virus could not replicate in the chicken embryonic cells as described earlier (Anonymous, 1994).

The pH range from 5 to 7 did not modulate the HA activity of the virus stored for 45 days. However, pH 3 or pH 9 did not reduce the HA activity of the virus when stored for 15 days. But, each of the pH of the virus suspension gradually declined the HA titer of the virus when stored for more than 15 days. Similar results have been reported by Foni *et al.* (1989) and Quinn *et al*. (2002).

Storage temperature showed detectable reduction in HA activity of the virus. Freezing temperature (-20°C & -196°C) when exposed for more than 30 min resulted in complete loss of HA activity of the virus. Freezing and thawing might be responsible for degradation of the virus haemagglutinins. However, non-freezing temperature had un-detectable effect on the HA activity of the virus. These results corroborate with the observations of Gorden and Angrick (1986) that the virus remains active for 12 months of storage at 4°C. However, growth of psychrophilic bacteria at this temperature may reduce the HA activity of the virus during storage. Addition of antimicrobial agents (formaldehyde, antibiotics, or sodium azide) in the virus suspension during storage may enhance the keeping quality of the stored HA virus (diagnostic).

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(Received 07 February 2006; Accepted 17 June 2006)