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Parvoviruses in Dogs

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Abstract: Canine parvovirus (CPV) is one of the most important endemic contagious enteric viral diseases infecting dogs especially puppies and characterized by clinical gastroenteritis. The virus affects dogs' gastrointestinal tracts and is spread by direct dog-to-dog contact and contact with contaminated feces (stool), environments, or people. The virus is resistant to heat, cold, humidity and drying and can survive in the environment for long periods of time. In Egypt, the virus was initially diagnosed in 1982 and aggressively attacks puppies and dogs leading to high mortality. There are no specific treatment for such disease. Moreover, reports of infections of vaccinated dogs has been recorded due to progressive evolving of antigenic variants which require an extensive revision of the vaccination programs and an update of the viral strains contained in the commercially available products as well as updating molecular methods to be able to detect and characterize the new CPV antigenic variants.

Key words: Parvovirus · CPV · Canine parvovirus type 2 · CPV-2

INTRODUCTION

Canine parvovirus (CPV) is one of the most important endemic contagious enteric viral diseases infecting dogs especially puppies and characterized by clinical gastroenteritis. Canine parvo virus type 2 (CPV-2) appeared in the late 1970s and became distributed all over the word within dog population within 2 years [1]. Unfortunately research articles regarding canine parvovirus are few in and there are a lot of reports concerning the infection of vaccinated dogs which urge further investigation about the efficacy of the available vaccine.

Genetic Evolution of Canine Parvovirus: Although CPV is a DNA virus, its genomic substitution rate is approximately 10-4 per site per year, which is similar to RNA viruses [2]. In 1978, CPV-2 was first identified from outbreaks in canines in the United States and Australia [3, 4]; it was then reported in many countries during 1978 and 1979 [5]. CPV-2 is closely related to feline parvovirus (FPV); therefore, it is assumed to be a host variant of FPV [3, 6]. CPV-2 was named to distinguish and differentiate it from an unrelated canine minute virus (CPV-1) [7]. In the 1980s, the original CPV-2 type was completely replaced by two new antigenic variants in canines, which were termed CPV types 2a (CPV-2a) and 2b (CPV-2b), where there is change to Asp or Glu in the VP2 residue 426 were detected in virus variants termed CPV-2b and -2c due to change in host range. CPV-2 is extremely contagious, causing high morbidity with increased incidence in closed area [8, 9]. In 2000, CPV-2c with Asp426Glu substitution was reported in Italy [10]. With its antigenicity constantly drifting, an increasing number of further mutations of VP2 have been described and various viral mutants have been named [11-13]. However, the nomenclature of these variants is inconsistent and confusing.

Virus Structure: Canine parvovirus (CPV) belongs to the genus *Protoparvovirus* and the family Parvoviridae [14]. CPV is a non-enveloped DNA virus with an approximately 5000-nucleotide, single-stranded DNA genome containing two open reading frames (ORFs). The first ORF encodes two non-structural proteins, NS1 and NS2. The second ORF encodes two structural proteins, VP1 and VP2 [15]. VP1 and VP2 each encode parts of the viral capsid, which is assembled from 54 copies of VP2 and 6 copies of VP1 [16]. VP2, the major capsid protein, is also the major antigenic protein and determines viral tissue tropism and host range [17, 18]. NS1, a pleiotropic nuclear phosphoprotein, plays an essential role in viral replication and is responsible for inducing cell apoptosis [19, 20].

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Virus Replication: Virus replication takes place in the cell nuclei and requires rapidly dividing cells of fetuses and newborns or of hematopoietic and intestinal tissues of young and adult animals. Their replication in vivo is rarely associated with the appearance of nuclear inclusion bodies, whereas the cytopathic effect induced in vitro is not always evident. All parvoviruses are highly stable in the environment, as they are extremely resistant to pH and temperature changes and to treatment with lipid solvents, trypsin and most disinfectants. Virions can be inactivated by formalin, sodium hypochlorite, beta propiolactone, hydroxylamine, oxidizing agents and ultraviolet irradiation. Several parvoviruses are able to agglutinate erythrocytes of different mammal and bird species and some diagnostic tests for parvovirus infections rely on this hemagglutination activity [21].

Disease Clinical Signs and Mode of Infection: The disease is characterized by a rapid clinical course with death that can often occur 2-3 days after onset of signs in non-protected hosts and puppies [22]. It can affect dogs at any age, but severe infection is most common in puppies between 6 weeks and 6 months of age [23]. All breeds are susceptible to the disease such as Rottweilers, Doberman Pinschers, English Springer Spaniels, American Pit Bull Terriers and German Shepherd. On the other hand, the mixed breeds are described to be less susceptible than many pure-breeds [24]. The infection is generally attained by the fecal-oral route through the contact with feces from infected dogs or contaminated surfaces. The virus is readily transmitted from place to place on the hair or feet of dogs or via contaminated cages, shoes, or other objects. It is resistant to heat, cold, humidity and drying and can survive in the environment for long periods of time. Once the virus enter the body, it affects mainly tissues, such as the lymphoid tissues, intestinal epithelium and bone marrow, as well as the heart in neonatal puppies. Following an incubation period of 3-7 days, the disease can be characterized by two clinical forms, the enteric form that comprises vomiting, hemorrhagic diarrhea, depression, loss of appetite, fever and dehydration in younger dogs [25]. Myocarditis may be seen after infection of neonatal puppies, where the clinical signs are seen a number of weeks after infection. CPV infection is also characterized by a drop in the white blood cell counts as a result of the infection of the bone marrow infection and other lymphoid tissues [3, 14].

Laboratory Diagnosis: CPV recognizes as target tissues for viral replication the intestinal crypts and the lymphoid organs, but the virus can spread to all tissues [26] including the brain [27, 28] After penetration through the oronasal route, the virus replicates in gastroenteric associated lymphoid tissues and is disseminated by infected leukocytes to the germinal epithelium of the crypts of the small intestine, causing diarrhea. Infection of leukocytes, mainly circulating and tissue-associated lymphocytes, induces acute lymphopenia (often associated with neutropenia) [26]. In 2-3-week-old seronegative pups, CPV is also able to replicate in cardiac cells inducing a fatal myocarditis. However, this form is no longer observed as almost all young pups are protected by maternally derived antibodies (MDA) [29]. The most characteristic clinical form induced by CPV is represented by hemorrhagic enteritis, the extent of which is often dependent on the MDA titers of the infected pups at the moment of infection. Clinical signs occur after an incubation period of 3-7 days and consist of anorexia, depression, vomiting and mucoid or bloody diarrhea, frequently dehydration and fever. Leukopenia is a constant finding, with white blood cell (WBC) counts dropping below 2000-3000 cells/mL of blood. However, total WBC counts may be even within normal ranges due to concomitant virus-induced lymphopenia and neutrophilia consequent to infections by opportunistic bacteria. Concurrent pulmonary infections may lead to the onset of respiratory distress. Subclinical and in-apparent infections are frequently detected, mainly in pups with intermediate MDA titers and in adult dogs [30]. The mortality rates can be high (up to 70%) in pups, but are usually less than 1% in adult dogs. Hemorrhagic enteritis of the small intestine and enlargement of mesenteric lymph nodes and Peyer's patches are the main gross lesions observed in dogs that die as a consequence of CPV infection. Histopathologically, the small intestine is affected by multifocal crypt necrosis and intranuclear inclusion bodies, whereas extensive depletion of lymphocytes is seen in Peyer's patches, lymph nodes, spleen and thymus. A rapid diagnosis of CPV infection is especially important in kennels and shelters in order to isolate infected dogs and prevent secondary infections of susceptible contact animals. Clinical diagnosis is indecisive and several other viral pathogens may cause diarrhea in dogs, such as coronaviruses, adenoviruses, morbilliviruses, rotaviruses, reoviruses, noroviruses [29]. Thus, a suspect clinical case should always be confirmed by laboratory tests. Several methods have been

developed for the laboratory diagnosis of CPV infection, which is usually carried out on the feces (or intestinal contents if the animal is dead) of affected dogs. In the late stages of infection, EDTA-blood samples have been proven to be more useful for the diagnosis as CPV viremia is exceptionally long lasting [31]. Tests detecting viral antigens by means of antibody-based methods are suitable for CPV diagnosis in the veterinary practice and represent the only tests available in the field [29]. However, their sensitivity, like other traditional diagnostic methods, has been proven inferior to molecular assays. An immuno-chromatographic (IC) test 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% [32]. The poor sensitivity of the IC 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 [33]. A more recent study compared the performances of three different commercially available, antibody-based tests for rapid detection of CPV antigens with PCR and immunoelectron microscopy, confirming the high specificity and low sensitivity of the antigen-detection kits [34]. Taking advantage on the close genetic and antigenic relationship among carnivore parvoviruses, in-house test systems developed for CPV diagnosis are able to detect also FPV [35]. Surprisingly, some concerns have been expressed about the ability of in-hospital rapid parvovirus tests to recognize efficiently the new variant CPV-2c. Those concerns took into account the circumstantial evidence that the increase in rapid test failure paralleled the emergence and spreading of CPV-2c [36]. Almost simultaneously, it was recommended to test the MAbs contained in the in-house assays against the additional mutations detected in strains currently circulating [37]. However, by IC testing of including 100 samples containing the recently identified CPV-2c, the detection rate of this variant was similar to those of CPV-2a/CPV-2b, thus dissipating any previous concerns about the hypothesized but never demonstrated less efficiency of the test in detecting the new variant [38]. Alternative techniques, such as hemagglutination (HA) and virus isolation (VI), can be carried out only in specialized laboratories. For a clear reading of the HA test, good quality erythrocytes should be ensured since the test is affected by an altered coefficient of erythrocyte sedimentation [33]. Furthermore, CPV-2 strains lacking HA activity have been reported [39]. Nevertheless, the HA test carried out in a 96- well plate format allows rapid processing of many samples. Results are read after only 4 h. VI requires the availability of cell cultures that can be propagated only in laboratories with specialized personnel and a cell culture capability. Moreover, VI is time-consuming; it requires a long incubation period (5-10 days) and additional testing by immunofluorescence or HA in order to detect viral antigens. The main disadvantage of HA and VI, however, is the low sensitivity, most likely due to antibodies in the intestinal lumen of the infected dogs which may bind virions and prevent both HA and viral attachment to cell receptors [33]. On the other hand, molecular methods are generally not affected by the host immune response, they are generally time consuming, labour-intensive and need the expertise of specialists. A nucleic acid hybridization assay was available since early 1990s. Subsequently, several PCR assays were developed that displayed increased sensitivity and specificity in comparison with traditional methods [33]. A loop-mediated isothermal amplification assay has been also proposed in recent years as an alternative to PCR-based methods. However, none of these nucleic-acid detection methods were designed to be quantitative, although they are time consuming and contain a certain risk of carryover contamination, especially when a high sample throughput is required. A real-time PCR assay based on the TaqMan technology was developed for rapid, specific and sensitive detection of CPV DNA [40, 41]. Real-time PCR has several advantages over conventional PCR, allowing a large increase in throughput and enabling simultaneous processing of several samples. Real-time PCR is run in a 96-well format and many of the steps in the assay are automated. Because of the inexpensive and quick method used for DNA preparation, based on boiling of fecal homogenates, the total time requested for analysis of 20-30 samples was about 6 and 3 h for conventional and real-time PCR, respectively [42]. The high sensitivity and reproducibility of the real-time PCR assay may allow for identification of dogs shedding CPV at low titers in their feces, helping to adopt adequate measures of prophylaxis to prevent CPV infection, especially in kennels and shelters, where this virus is often responsible for dramatic epizootics. A SYBR Green-based real-time PCR assay was proposed as an alternative method to the TaqMan technology, displaying the same detection limit (10 copies of viral DNA) [43].

Control: Prophylaxis of CPV infection relies mainly on extensive vaccination. Since inactivated vaccines are able to induce only short-term immunity, modified live virus

(MLV) vaccines are widely used. These vaccines, prepared by using either the original type CPV-2 or its variant CPV-2b, are highly effective, being able to protect dogs against parvo-viral disease as well as infection and almost completely safe, as post-vaccinal reactions are very rarely observed. A recent study showed that most dogs developing parvovirus-like diarrhea after vaccination were infected by the field virus alone or with the attenuated vaccine virus. The primary causes of failure of CPV vaccination are interfering levels of MDA that are transmitted by bitches to their offspring through colostrum and, at a lesser extent, milk. Thus, in order to avoid the interference with active immunization, vaccines should be administered to pups only after waning of MDA [29]. Different strategies have been proposed to overcome the MDA interference, including high-titer vaccines and intranasal vaccination. The Vaccination Guidelines Group of the World Small Animal Veterinary Association also recommends delaying finish of primary CPV vaccination course to 14-16 weeks of age to ensure protection even in pups with long-lasting MDA. In addition, there are some concerns about the complete efficacy of type 2-based vaccines against the antigenic variants. Good hygiene is also important to prevent infection. CPV is a resilient virus and can live on some surfaces for more than a year. A bleach solution containing 1 part bleach and 30 parts water (1:30) is necessary to kill the virus. Care should be exercised when exposing a puppy or dog to areas where other dogs congregate, such as parks, highway rest areas, obedience classes, kennels and grooming facilities. Owners should prevent dogs from coming into contact with the feces of other dogs. Proper disposal of waste matter can help prevent the spread of CPV infection [36, 30].

Treatment: Treatment for canine parvovirus is supportive and the goals are to relieve symptoms and prevent complications. Because the disease progresses so quickly, it is important to begin treatment as soon as possible. In most cases, hospitalization is required. Treatment usually involves intravenous (IV) fluids, anti-nausea medications and antibiotics to prevent secondary infections. Infected dogs should be kept warm and away from other dogs and activity should be restricted. Treatment for CPV is not always successful, even when it is started promptly. In dogs that recover from infection, improvement may be seen in 2 or 3 days. Recent trails to treat infection by using interferon omega (feline interferon) which showed promising results in treatment of beagle pups (8-9 weeks) [44]. Other clinical trials of transplant microbiota into infected puppies [45].

Disease in Egypt: In Egypt, the virus was in the beginning reported, in 1982, in military police dogs showing clinical manifestations and pathological features of CPV outcomes [46]. In 2018, genotypes 2a and 2b were identified using genetic characterization with special reference to multiple mutations in genotype 2b [47]. Genotype 2b is circulating in Egypt with successful isolation of virus on Vero cells [48].

CONCLUSIONS

Since FPV's first identification in 1920 CPV has progressively evolved showing genomic substitution rates similar to those of RNA viruses. Such an evolution has led to the emergence of antigenic variants that seem to be more virulent than the original type. In contrast, despite some circumstantial reports claiming a higher pathogenicity of CPV- 2c, there is no available data for a different virulence between the variants. Without a doubt, apart from well-documented case reports, experimental data and field observations seem to suggest that the great majority of infections still occur in the puppies about the time when MDA fade and the animals become susceptible to any strain of virus. In conclusion, continuous evolution of CPV through accumulation of point mutations in the viral genome presents two different, but equally important, implications: (i) the emergence of new different biological and antigenic variants require an extensive revision of the vaccination programs and an update of the viral strains contained in the commercially available products; (ii) updating molecular methods to be able to detect and characterize the new CPV antigenic variants. Thus, a continuous epidemiological surveillance is needed to detect new CPV variants potentially escaping the host immune system and detection methods.

REFERENCES

1. Carman, P.S. and R.C. Povey, 1985. Pathogenesis of canine parvovirus-2 in dogs: haematology, serology and virus recovery. Res. Vet. Sci., 38(1): 134-140.
2. Shackelton, L.A, C.R. Parrish, U. Truyen and E.C. Holmes, 2005. High rate of viral evolution associated with the emergence of carnivore parvovirus. Proc. of the Nat.l Acad. of Sci. of the USA, 102(2): 379-384.
3. Appel, M.J., B.J. Cooper, H. Greisen, F. Scott, L.E. Carmichael, 1979. Canine viral enteritis. I. Status report on corona- and parvo-like viral enteritides. The Cornell Veterinarian, 69(3): 123-133.

4. Appel, M.J.G. and L.E. Carmichael Inventors, 1980. Google Patents, assignee. Canine parvovirus vaccine US patent, 4(193):991-998.
5. Carmichael, L.E. and L.N. Binn, 1981. New enteric viruses in the dog. Adv. in vet. Sci. and Comp. Med., 25(1): 1-37.
6. Parrish, C.R., 1990. Emergence, natural history and variation of canine, mink and feline parvoviruses. Adv. in Virus Res., 38: 403-450.
7. Binn, L.N., E.C. Lazar, G.A. Eddy and M. Kajima, 1970. Recovery and characterization of a minute virus of canines. Inf. and Imm., 1(5): 503-508.
8. Parrish, C.R., P. Have, W.J. Foreyt, J.F. Evermann, M. Senda and L.E. Carmichael, 1988. The global spread and replacement of canine parvovirus strains. The J. Gen. Virol., 69(5): 1111-1116.
9. Parrish, C.R., C.F. Aquadro, M.L. Strassheim, J.F. Evermann, J.Y. Sgro and H.O. Mohammed, 1991. Rapid antigenictype replacement and DNA sequence evolution of canine parvovirus. J. Virol.; 65(12): 6544-6552.
10. Buonavoglia, C., V. Martella, A. Pratelli, M. Tempesta, A. Cavalli and D. Buonavoglia, 2001. Evidence for evolution of canine parvovirus type 2 in Italy. The J. Gen. Virol.; 82(12): 3021-3025.
11. Ikeda, Y., M. Mochizuki, R. Naito, K. Nakamura, T. Miyazawa and T. Mikami, 2000. Predominance of canine parvovirus (CPV) in unvaccinated cat populations and emergence of new antigenic types of CPVs in cats. Virol., 278(1): 13-19.
12. Kang, B.K., D.S. Song, C.S. Lee, K.I. Jung, S.J. Park and E.M. Kim, 2008. Prevalence and genetic characterization of canine parvoviruses in Korea. Virus Gen., 36(1): 127-133.
13. Ohshima, T., M. Hisaka, K. Kawakami, M. Kishi, Y. Tohya and M. Mochizuki, 2008. Chronological analysis of canine parvovirus type 2 isolates in Japan. The J. Vet. Med. Sci., 70(8): 769-775.
14. Kelly, W., 1978. An enteric disease of dogs resembling feline panleucopaenia. Aust. Vet. J., 54(12): 593-601.
15. Reed, A.P., E.V. Jones and T.J. Miller, 1988. Nucleotide sequence and genome organization of canine parvovirus. J. Virol., 62(1): 266-276.
16. Vihinen-Ranta, M., D. Wang, W.S. Weichert and C.R. Parrish, 2002. The VP1 N-terminal sequence of canine parvovirus affects nuclear transport of capsids and efficient cell infection. J. Virol., 76(4):1884-1891.
17. Hueffer, K., J.S. Parker, W.S. Weichert, R.E. Geisel, J.Y. Sgro and C.R. Parrish, 2003. The natural host range shift and subsequent evolution of canine parvovirus resulted from virus-specific binding to the canine transferrin receptor. J. Virol., 77(3): 1718-1726.
18. Nelson, C.D., L.M. Palermo, S.L. Hafenstein and C.R. Parrish, 2007. Different mechanisms of antibody-mediated neutralization of parvoviruses revealed using the Fab fragments of monoclonal antibodies. Virol., 361(2): 283-293.
19. Saxena, L., G.R. Kumar, S. Saxena, U. Chaturvedi, A.P Sahoo and L.V. Singh, 2013. Apoptosis induced by NS1 gene of Canine Parvovirus-2 is caspase dependent and pp: 53 independent. Virus Res., 173(2): 426-430.
20. Gupta, S.K., A.P. Sahoo, N. Rosh, R.K. Gandham, L. Saxena and A.K. Singh, 2016. Canine parvovirus NS1 induced apoptosis involves mitochondria, accumulation of reactive oxygen species and activation of caspases. Virus Res., 213(1): 46-61.
21. Muzyczka, N. and K.I. Berns, 2001. Parvoviridae: The Viruses and Their Replication. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Vorology. 4th ed. Lippincott Williams & Wilkins, Philadephia, PA, pp: 2327-2359.
22. Parrish, C.R., 1995. Pathogenesis of feline panleukopenia virus and canine parvovirus. Baillieres Clin. Haematol., 8(5): 57-71.
23. Houston, D.M., C.S. Ribble and L.L. Head, 1996. Risk factors associated with parvovirus enteritis in dogs: 283 cases (1982-1991). J Am. Vet. Med. Assoc., 208(1): 542-546.
24. Glickman, L.T., L.M. Domanski, G.J. Patronek and F. Visintainer, 1985. Breed-related risk factors for canine parvovirus enteritis. J. Am. Vet. Med. Assoc., 187(4): 589-594.
25. Hoelzer, K., L.A. Shackelton, E.C. Holmes and C.R. Parrish, 2008. Within-host genetic diversity of endemic and emerging parvoviruses of dogs and cats. J. Virol., 82(6): 11096-11105.
26. Pollock, R.V., 1982. Experimental canine parvovirus infection in dogs. Cornell. Vet., 72(2): 103-119.
273. Elia, G., A. Cavalli, C. Desario, E. Lorusso, M.S. Lucente, N. Decaro, V. Martella and C. Buonavoglia, 2007. Detection of infectious canine parvovirus type 2 by mRNA real-time RT-PCR. J. Virol. Meth., 146(1): 202-208.

28. Decaro, N., F. Cirone, C. Desario, G. Elia, E. Lorusso, M.L. Colaianni, V. Martella and C. Buonavoglia, 2009. Severe parvovirus in a 12-yearold dog that had been repeatedly vaccinated. Vet. Rec., 164(4): 593-595.
29. Greene, C.E. and N. Decaro, 2012. Canine Viral Enteritis. In: Greene, C.E. (Ed.), Infectious Diseases of the Dog and Cat, 4th ed. WB Saunders, Philadelphia, PA.
30. Decaro, N., C. Desario, M.S. Lucente, F. Amorisco, M. Campolo, G. Elia, A. Cavalli, V. Martella and C. Buonavoglia, 2008. Specific identification of feline panleukopenia virus and its rapid differentiation from canine parvoviruses using minor groove binder probes. J. Virol. Methods, 147(5): 67-71.
31. Decaro, N., M. Campolo, C. Desario, G. Elia, V. Martella, E. Lorusso and C. Buonavoglia, 2005. Maternally-derived antibodies in pups and protection from canine parvovirus infection. Biologicals, 33(1): 259-265.
32. Faz, M., J.S. Martínez, I. Quijano-Hernández and R. Fajardo, 2017. Reliability of clinical diagnosis and laboratory testing techniques currently used for identification of canine parvovirus enteritis in clinical settings. J. Vet. Med. Sci., 79(1): 213-217.
33. Desario, C., N. Decaro, M. Campolo, A. Cavalli, F. Cirone, G. Elia, V. Martella, E. Lorusso, M. Camero and C. Buonavoglia, 2005. Canine parvovirus infection: which diagnostic test for virus? J. Virol. Methods, 121(3): 179-185.
34. Schmitz, S., C. Coenen, M. Ko¨ nig, H.J. Thiel and R. Neiger, 2009. Comparison of three rapid commercial canine parvovirus antigen detection tests with electron microscopy and polymerase chain reaction. J. Vet. Diagn. Invest., 21(6): 344-345.
35. Neuerer, F.F., K. Horlacher, U. Truyen and K. Hartmann, 2008. Comparison of different in-house test systems to detect parvovirus in faeces of cats. J. Feline. Med. Surg., 10(1): 247-251.
36. Kapil, S., E. Cooper, C. Lamm, B. Murray, G. Rezabek, L. 3rd Johnston, G. Campbell and B. Johnson, 2007. Canine parvovirus types 2c and 2b circulating in North American dogs in 2006 and 2007. J. Clin. Microbiol., 45(3): 4044-4047.
37. Hong, C., N. Decaro, C. Desario, P. Tanner, M.C. Pardo, S. Sanchez, C. Buonavoglia and J.T. Saliki, 2007. Occurrence of canine parvovirus type 2c in the United States. J. Vet. Diagn. Invest., 19(1): 535-539.
38. Decaro, N., C. Desario, M.J. Beall, A. Cavalli, M. Campolo, A.A. Dimarco, F. Amorisco, M.L. Colaianni and C. Buonavoglia, 2010. Detection of canine parvovirus type 2c by a commercially available in-house rapid test. Vet. J., 184(11): 373-375.
39. Cavalli, A., G. Bozzo, N. Decaro, A. Tinelli, A. Aliberti and D. Buonavoglia, 2001. Characterization of a canine parvovirus strain isolated from an adult dog. New Microbiol., 24(3): 239-242.
40. Decaro, N., C. Desario, M. Campolo, G. Elia, V. Martella, D. Ricci, E. Lorusso and C. Buonavoglia, 2005. Clinical and virological findings in pups naturally infected by canine parvovirus type 2 Glu-426 mutant. J. Vet. Diagn. Invest., 17(5): 133-138.
41. Ortegaa, A.F., J.S. Martínez-Castan˜ edaa, L.G. Bautista-Gómezb, R.F. Mun˜ oza and I.Q. Hernándezc, 2017. Identification of co-infection by rotavirus and parvovirus in dogs with gastroenteritis in Mexico. Brazilian J. Micro., 48(3): 769-773.
42. Decaro, N., G. Elia, V. Martella, C. Desario, M. Campolo, L. Di Trani, E. Tarsitano, M. Tempesta and C. Buonavoglia, 2005. A real-time PCR assay for rapid detection and quantitation of canine parvovirus type 2 DNA in the feces of dogs. Vet. Microbiol., 105(1): 19-28.
43. Kumar, M. and S. Nandi, 2010. Development of a SYBR Green based real-time PCR assay for detection and quantitation of canine parvovirus in faecal samples. J. Virol. Methods, 169(1): 198-201.
44. Martin V., W. Najbar, S. Gueguen, D. Grousson, H.M. Eun, B. Lebreux and A. Aubert, 2002. Treatment of canine parvoviral enteritis with interferon-omega in a placebo-controlled challenge trial. Vet. Micro., 89(2-3): 115-127.
45. Pereira, G.Q., L.A. Gomes, I.S. Santos, A.F. Alfieri, J.. Weese and M.C. Costa, 2018. Fecal microbiota transplantation in puppies with canine parvovirus infection. J. Vet. Intern. Med., 32(1): 707-711.
46. Bucci, T.J., A.M. Botros and M. El-Molla, 1982. Canine parvovirus infection: A brief review & report of fist cases in Egypt. J. Egypt. Vet. Med. Associ., 42(1): 21-25.
47. AL-Hosary, A.A., 2018. Detection and molecular characterization of parvovirus serotypes in Egypt. J. Adv. Vet. Res., 8(4): 79-83.
48. Soliman, R.M., N.M. Baker, M.Y. Nasr and M.H. Khodeir, 2018. Clinical, virological and molecular characterization of canine parvo virus in dogs. Eur. J. Pharm. Med. Res., 5(4): 525-535.