Silver nanoparticles composition for treatment of distemper in dogs

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Abstract: Canine distemper is caused by the canine distemper virus (CDV), a paramyxovirus of the genus Morbillivirus, with a worldwide distribution. Canine distemper is a multisystem disease, potentially fatal. Affected organisms may develop clinical or subclinical infections, among them respiratory, gastrointestinal and of the nervous system. In the present work, we use a veterinary pharmaceutical composition of silver nanoparticles (AgNPs) for the treatment of distemper in dogs with neurological and non-neurological symptoms. AgNPs treatment of animals with non-neurological distemper provides a very high rate of recovery without sequelae. However, in dogs with neurological symptoms, AgNPs treatment was not able to reverse the disease.

Keywords: AgNPs treatment; canine distemper virus; CDV; distemper virus; silver nanoparticles.

Reference to this paper should be made as follows: Bogdanchikova, N., Vázquez-Muñoz, R., Huerta-Saquero, A., Pena-Jasso, A., Aguilar-Uzcanga, G., Picos-Díaz, P.L., Pestryakov, A., Burmistrov, V., Martynyuk, O., Luna-Vázquez-Gómez, R. and Almanza, H. (2016) 'Silver nanoparticles composition for treatment of distemper in dogs', *Int. J. Nanotechnol.*, Vol. 13, Nos. 1/2/3, pp.227–237.

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This paper is a revised and expanded version of a paper entitled 'Silver nanoparticles composition for treatment of distemper in dogs' presented at *IV Russian-Mexican workshop on Nanoparticles, Nanomaterials and Nanoprocessing*, CNYN-UNAM, Ensenada, Mexico, 6–9 May, 2014.

1 Introduction

Distemper is a highly contagious viral disease that affects animals of the families Canidae, Mustelidae, Mephitidae, Hyaenidae, Ailuridae, Procyonidae, Pinnipedia, Viverridae and Felidae. Of those families most common animals by their relationship with humans are dogs and in recent years ferrets [1]. Canine distemper is caused by the canine distemper virus (CDV), a paramyxovirus of the genus Morbillivirus, with a worldwide distribution. It occurs as a multisystem disease, potentially fatal [2].

Affected organisms may develop clinical or subclinical infections, mainly with respiratory, gastrointestinal and nervous system affections, which vary greatly depending on the viral strain, infective dose and immune response of each patient. Nowadays, there is no specific antiviral treatment for CDV, and infected organisms usually suffer complications from bacterial infections. The early destruction of lymphocytes mediated by the virus results in immunosuppression of infected animals [3]. Canine distemper transmission occurs by respiratory secretions, eye secretions, urine and faeces.

Contact between infected animals keeps the virus in the canine population in general and the birth of pups provides a group susceptible to be infected. Dogs that surpass distemper infection are immune against the virus throughout their life. Immunity induced by distemper vaccination is prolonged, but does not protect dogs for life. Dogs that do not receive regular vaccinations can lose their protection and become infected after high-stress events, immunosuppression and exposure to highly polluted environments. Traditional treatment involves: antiviral agents, broad spectrum antibiotics which prevent bacterial complications, cleaning of nasal and ocular secretions, sedatives and anticonvulsants, vitamin complex and immune system stimulants. Frequency and dosage depend on type and intensity of distemper. Distemper treatment can be costly and prolonged. Additionally, life expectancy is very low, with over 85% of deaths. Among antiviral agents, Ribavirin (RBV) causes a dose- and time-dependent decrease in accumulation of CDV RNA when added after virus adsorption. RBV was highly effective in preventing CDV replication at low concentrations with 50% virus-inhibitory concentrations ranging from 0.02 mM to 0.05 mM. This data suggest RBV as a promising tool for the therapy of CDV in dogs [4].

Dal Pozzo et al. demonstrated in vitro antiviral activity of the 5-ethynyl-1-beta-D-ribofuranosylimidazole-4-carboxamide (EICAR) [5]. They found EICAR was more active than RBV against CDV replication. Antiviral activity of flavonoids quercetin, morin, rutin and hesperidin, and phenolic cinnamic, trans-cinnamic and ferulic acids were useful at early steps of viral infection [6]. Proanthocyanidin A2 isolated from the bark

of *Aesculus hippocastanum*, showed potential usefulness as an anti-CDV compound inhibiting viral replication in vitro [7]. Acetanilide derivatives inhibit measles virus (MV)-, CDV- and Nipah virus (NiV)-induced membrane fusion in tissue culture experiments including highly sensitive primary cells [8]. Recently, monovalent and multivalent DNA-based vaccines against canine distemper were designed and showed efficacy inducing humoral immune response in mice and dogs [9]. On the other hand, silver nanoparticles have emerged as novel antiviral agents based on their unique chemical and physical properties [10]. Silver nanoparticles have been studied for their antimicrobial potential against bacteria, but have also proven to be active against several types of viruses including human immunodeficiency virus [11,12], hepatitis B virus [13] and herpes simplex virus [14], among others.

The mechanism of action of AgNPs as antiviral agent has been studied against several enveloped viruses [15]. It has been proposed that silver nanoparticles interact with the HIV-1 virus via preferential binding to the gp120 glycoprotein knobs. Owing to this interaction, silver nanoparticles inhibit the virus from binding to host cells [11]. Cell-based fusion assay showed that AgNPs inhibit HIV-1 infection by blocking the viral entry, particularly the gp120-CD4 interaction [12]. Other studies also showed that AgNPs inhibit arenavirus replication during the early phases of viral replication [16]. AgNPs inhibits influenza virus interfering with the fusion of the viral membrane, inhibiting viral penetration into the host cell [17]. CDV has an enveloped virion containing RNA [1]. It has two glycoproteins (haemagglutinin H and fusion protein F). CDV uses protein H for attachment to receptors on the cell in the first step of infection [18]. After attachment, F protein promotes fusion of cell membrane with viral envelope [19]. Taken together, we hypothesised whether AgNPs could interfere with CDV infection in dogs to overcome disease. Here we report the successful use of veterinary pharmaceutical composition of silver nanoparticles (AgNPs) for the treatment of canine distemper.

2 Experimental

Dogs recruitment. Dogs brought by their owners to receive veterinary care and who were diagnosed with CDV were included in the AgNPs treatment with the consent of their owners. A group of dogs whose owners did not approve AgNPs treatment were treated with antibiotics, anti-inflammatories, antiemetic, but not antiviral and are presented here as controls.

Evaluation of dogs with CDV. Dogs were evaluated before and during treatment: weight, age, breed and sex, as well as symptoms related to distemper disease (neurological, gastrointestinal, ocular or respiratory damage) were registered. Canine IgM antibody to distemper virus was quantified by CDV IFA antibody kit (Fuller laboratories) according to manufacturer instructions. Dogs were classified first by distemper related symptoms. Among them, IgM tested positive for distemper were grouped as follows:

- dogs with non-neurological symptoms variants: gastrointestinal, respiratory, ocular; + AgNPs treatment
- dogs with neurological symptoms; + AgNPs treatment
- dogs with any distemper symptoms that do not receive AgNPs treatment.

Silver nanoparticles preparation. Silver nanoparticles solution for treatment was prepared from concentrated solution and directly before consumption. Method of dilution is very simple and includes the addition of low amounts of concentrated solution into water. AgNPs concentrated solution is stable for two years (kept in refrigerator at 4°C). After two years, biological activity decreases by 15%. The date of expiration was determined by measuring AgNPs size, their agglomeration evaluated by physicochemical methods and by their antimicrobial activity tested with different biological models (Gram-Positive and Gram-negative bacteria and fungi) (Data not shown).

For canine distemper treatment, we used silver nanoparticles containing 6% of metallic silver and 94% of polyvinylpyrrolidone solution (PVP, weight/volume), with a particle size in the range from 0.5 nm to 100 nm. Veterinary composition of silver nanoparticles used had a concentration of 1.8 mg/ml. At lower concentrations (below 1.8 mg/ml) of AgNPs, healing is slower and less efficient, while at higher concentrations, there is a risk of nanoparticle accumulation in different organs and tissues that could negatively influence the animal health. For administration, AgNPs were diluted in bottled drinking water.

Dosage. Dose and time of administration were as follows:

- dose of 1 ml of AgNPs solution per kilogram for a period between 7 and 15 days for dogs with non-neurological distemper symptoms (1.8 mg of AgNPs/kg per day)
- twice daily administration of 1 ml of AgNPs solution per kilogram for treatment of very sick animals with neurological damage, once in the morning and once in the afternoon, during 25–28 days.

3 Results

Among dogs with non-neurological distemper treated with AgNPs, 90% recovered motility and appetite after seven days of application. However, there still remained some typical symptoms of the disease (Figure 1). Complete recovery was observed after 10 to advanced stage of the disease. Of nine dogs treated with AgNPs, eight survived without sequelae and one died for unknown causes (Table 1).

Table 2 shows the results of treatment of dogs with distemper with neurological symptoms. This group was treated with twice-daily administration of AgNPs.

In general, adult animals and those that were detected in the first stage of the disease showed better response to treatment than younger dogs and those that were treated in 15 days of treatment, when symptoms disappear and dogs are considered healthy (Table 1). During the third week treated dogs continued healthy without sequelae. Of 15 dogs treated, one dog (N-10) survived apparently without sequelae after 28 days of treatment; 12 died due to neurological distemper and two (N-1 and N-3) were euthanised at the request of their owners. Average value for IgM in dogs with non-neurological distemper (excluding N-7, which died) was 1 : 157 (±59 SE). Meanwhile the average for IgM values in dogs with neurological distemper (excluding N-10, which survived) was 1 : 66 (±18 SE). In the first case 90% of infected dogs recovered, meanwhile in the second case only 6% of infected dogs recovered without sequelae.

Among dogs with any distemper symptoms (neurological or non-neurological) that do not receive AgNPs treatment, all 16 died for distemper related complications (Table 3). Summary of results is shown in Figure 1.

Figure 1 Dogs affected with distemper were classified as non-neurological and neurological. Percentage of recovery among groups treated and non-treated with AgNPs

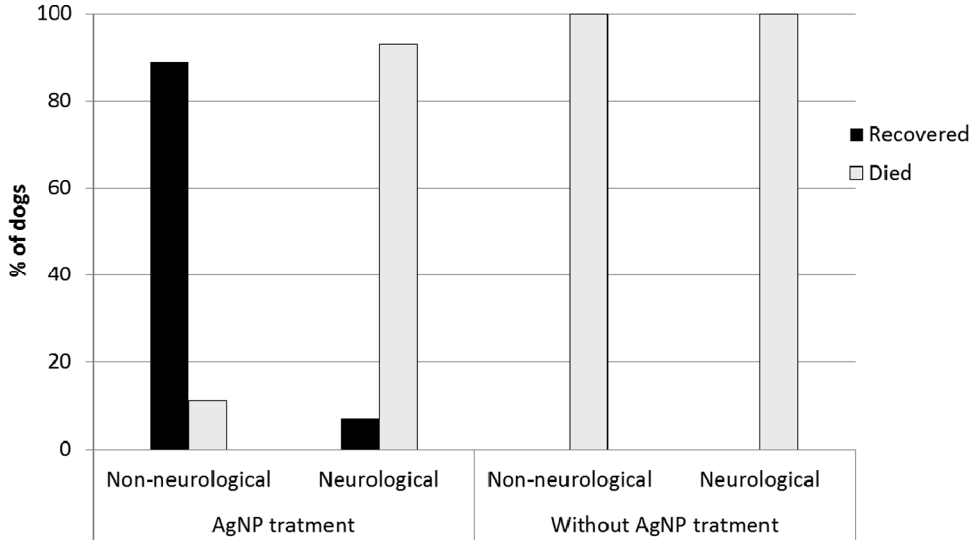

Table 1 Results of treatment of dogs with non-neurological distemper

No.	*Breed*	*Sex*	*Weight* (kg)	*Age, months*	*Symptoms*	*IgM value*	*Report*
1	Schnauzer	F	n/d	n/d	Gastrointestinal: diarrhoea, positive for Isospora. Respiratory: tracheobronchitis. Ocular: keratoconjunctivitis	1:80	Recovered without sequelae
2	Great Dane	F	13.4	3	Gastrointestinal: diarrhoea, positive for Giardia. Respiratory: Pneumonia.	1:40	Recovered without sequelae
3	Rottweiler	F	6	3	Gastrointestinal: diarrhoea and vomit. Respiratory	n/d	Recovered without sequelae
4	Mixed	M	6.2	12	Gastrointestinal: diarrhoea	1:320	Recovered without sequelae
5	Schnauzer	M	6	4	Respiratory	1:160	Recovered without sequelae
6	Mixed	F	17	48	Ocular: Eye injury. Depression, weight loss	1:60	Recovered without sequelae
7	Mixed	M	4	24	Respiratory	1:40	Died
8	Schnauzer	M	10.2	36	Gastrointestinal: Anorexia and depression	1:280	Recovered without sequelae
9	Labrador	M	30	24	Gastrointestinal, respiratory	1:160	Recovered without sequelae

Table 2 Results of dogs treatment with neurological symptoms

No.	*Breed*	*Sex*	*Weight* (kg)	*Age, months*	*Symptoms*	*IgM value*	*Report*
1	Poodle	M	4.3	48	Gastrointestinal: diarrhoea, vomit. Neurological: ataxia, mioclony, hyperparesthesia. Ocular	1:120	No recovered. Eutanasia
2	Belgian sheperd maronoise	F	18	7	Respiratory neurological	1:80	Died
3	Mixed	M	8	4	Neurological respiratoriy	1:160	No recovered. Eutanasia
4	Siberian Husky	M	6.8	3	Neurological	n/d	Died
5	Mixed	F	3	48	Neurological	1:40	Died
6	Labrador	M	7	4	Neurological gastrointestinal ocular	1:20	Died
7	Labrador	M	4	4	Sistemic, neurological,	1:40	Died
8	Boxer	F	13.4	4	Neurological	1:60	Died
9	Old English sheperd	F	7	4	Neurological gastrointestinal respiratory	1:80	Died
10	Poodle	F	5.2	12	Neurological, gastrointestinal	1:320	Recovered without sequelae
11	Poodle	F	2.5	48	Neurological, gastrointestinal cutaneus	1:40	Died
12	Poodle	M	3	6	Neurological	1:20	Died
13	Mixed	M	8	4	Neurological	1:160	Died
14	Poodle	M	6	24	Neurological	1:20	Died
15	Pug	M	3.7	3	Neurological	1:20	Died

Table 3 Group of dogs with distemper symptoms (neurological and non-neurological) non-treated with AgNPs

No.	*Breed*	*Sex*	*Age, months*	*Symptoms*	*IgM value*	*Report*
1	Pug	M	3.5	Respiratory	1:80	Died
2	Labrador	M	24	Neurological	1:40	Died
3	Mixed	M	12	Gastrointestinal, respiratory	1:320	Died
4	Chow-chow	M	5	Respiratory	1:60	Died
5	Belgian sheperd	F	8	Gastrointestinal	1:80	Died
6	Beagle	M	6	Gastrointestinal	1:40	Died
7	Poodle	F	36	Gastrointestinal	1:40	Died
8	Old English sheperd	F	4	Gastrointestinal	1:80	Died
9	Poodle	M	36	Gastrointestinal, neurological	1:20	Died
10	Mixed	F	24	Neurological	1:40	Died
11	German sheperd	M	12	Neurological	1:80	Died
12	Chihuahua	F	4	Respiratory	1:320	Died
13	Daschound	F	12	Gastrointestinal, respiratory	1:80	Died
14	Poodle	M	72	Gastrointestinal, respiratory	1:40	Died
15	Poodle	M	144	Neurological	1:80	Died
16	Poodle	M	2	Gastrointestinal, neurological	1:40	Died

4 Discussion

Canine distemper is an important worldwide disease that primarily affects dogs. Vaccine-based prophylaxis has greatly helped to keep distemper disease under control. Although, emergence of new CDV strains and trading between countries with low control of health standards increase importantly the number of CDV infections around the world. The development of new antiviral drugs could be an alternative for the short term; however, viral diversity is a limiting factor for the success of these substances. On the other hand, the ability of AgNPs as unspecific antiviral agent provides an alternative for the treatment of such infection. Currently, it is proposed that AgNPs act by interfering with viral infection, particularly during attachment and entry. AgNPs block the attachment and consequently the entry of virus into host cells as demonstrated for HIV infection [11] and influenza virus infection [17], among other studies. Functional groups on the virus surface are disturbed by AgNPs. Furthermore, the pharmaceutical formulation of AgNPs stabilised with PVP used in this work has shown very low toxicity in cultured cells and human lymphocytes as well as in experimental animal models (Balb/c mice and Wistar rats) (results will be published separately elsewhere).

Here, we report that treatment with AgNPs in animals with no neurological distemper (i.e., at early stages of disease) provides a very high rate of recovery without sequelae (over 90% of recovery, Figure 1). Virus-specific immunoglobulin M (IgM) persists for at least 3 months after infection and was used as a marker of recent CDV infection [20,21]. It is important to mention that higher IgM values for CDV found for the earlier stages of the disease favours recovery. For neurological stages immune response is overcome, which leads to treatment fail. This permits us to suggest that the higher IgM titre the higher probability of recovery. Two exceptional cases (N-7 of Table 1 and N-10 of Table 2) can be explained better taking into account IgM values rather than disease symptoms. For example, dog number 10 (Table 2) having an IgM titre of 1 : 320 and neurological diagnosis recovers in comparison with the rest of the cases whose IgM is below 1:160 due to its stronger immune response concomitantly to AgNPs treatment.

For studied AgNPs concentration and doses, silver nanoparticles treatment is successful in combination with a high immune response. In contrast, silver nanoparticles treatment (even applying double AgNPs doses) in combination with low immune response failed.

Also it is worth to mention that probably bioavailability of the silver nanoparticles is different for different tissue. This issue was not studied for applied type of silver nanoparticles yet. Nevertheless, we did not find any publication dedicated to the treatment of neurological diseases by any type of silver nanoparticles. It was revealed that AgNPs administered in a rat model with doses 30 mg/kg via intravenous and 50 mg/kg via intraperitoneal can cross the blood brain barrier [22]. In our case, for dogs with neurological symptoms applied dose was 1.8 mg/kg (twice a day), which is much lower than reported above and administered orally. So, further experiments with higher AgNPs doses and different administration routes are necessary to rule out neurological distemper can be treated successfully.

Although the use of our AgNPs is primarily directed to pets, treating distemper can be carried out in animals of the different families affected, which can be found in zoos, wild life reserves, or even as pets. Unfortunately, very sick animals with neurological symptoms showed no recovery after AgNPs treatment in the majority of cases.

5 Conclusions

In summary, veterinary composition of AgNPs stabilised with polyvinylpyrrolidone is useful for the treatment of distemper in its different variants, such as respiratory distemper, unapparent or acute distemper. The composition of AgNPs has no negative effects in treated animals, and treatment time for non-neurological distemper takes from 7 to 15 days. Owing to its effectiveness, low cost and easy handling, treatment with AgNPs composition presented here is an alternative in the treatment of distemper.

Acknowledgements

The authors acknowledge funding for this research by CONACYT Project No. 260409, PAPIIT-UNAM Projects IT200311 and IT200114-3 (Mexico) and by Russian Government Program 'Science' of Tomsk Polytechnic University, Grant No. 4.1187.2014/K.; to Myrna Romano for IgM measuring and J. Peralta and M. Sainz for technical assistance.

References

1 Martella, V., Elia, G. and Buonavoglia, C. (2008) 'Canine distemper virus', *Vet. Clin. North Am. Small Anim. Pract*., Vol. 38, No. 4, pp.787–797.

2 Carpenter, M.A., Appel, M.J., Roelke-Parker, M.E., Munson, L., Hofer, H., East, M. and O'Brien, S.J. (1998) 'Genetic characterization of canine distemper virus in Serengeti carnivores', *Vet. Immunol. Immunopathol.*, Vol. 65, Nos. 2–4, pp.259–266.

3 Carvalho, O.V., Botelho, C.V., Ferreira, C.G., Scherer, P.O., Soares-Martins, J.A., Almeida, M.R. and Silva Júnior, A. (2012) 'Immunopathogenic and neurological mechanisms of canine distemper virus', *Adv. Virol*., Vol. 2012, pp.1–10.

4 Elia, G., Belloli, C., Cirone, F., Lucente, M.S., Caruso, M., Martella, V., Decaro, N., Buonavoglia, C. and Ormas, P. (2008) 'In vitro efficacy of ribavirin against canine distemper virus', *Antiviral Res*., Vol. 77, No. 2, pp.108–113.

5 Dal Pozzo, F., Galligioni, V., Vaccari, F., Gallina, L., Battilani, M. and Scagliarini, A. (2010) 'Antiviral efficacy of EICAR against canine distemper virus (CDV) in vitro', *Res. Vet. Sci*., Vol. 88, No. 2, pp.339–344.

6 Carvalho, O.V., Botelho, C.V., Ferreira, C.G., Ferreira, H.C., Santos, M.R., Diaz, M.A., Oliveira, T.T., Soares-Martins, J.A., Almeida, M.R. and Silva Jr., A. (2013) 'In vitro inhibition of canine distemper virus by flavonoids and phenolic acids: implications of structural differences for antiviral design', *Res. Vet. Sci*., Vol. 95, No. 2, pp.717–724.

7 Gallina, L., Dal Pozzo, F., Galligioni, V., Bombardelli, E. and Scagliarini, A. (2011) 'Inhibition of viral RNA synthesis in canine distemper virus infection by proanthocyanidin A2', *Antiviral Res*., Vol. 92, No. 3, pp.447–452.

8 Singethan, K., Hiltensperger, G., Kendl, S., Wohlfahrt, J., Plattet, P., Holzgrabe, U. and Schneider-Schaulies, J. (2010) 'N-(3-Cyanophenyl)-2-phenylacetamide, an effective inhibitor of morbillivirus-induced membrane fusion with low cytotoxicity', *J. Gen. Virol*., Vol. 91, No. 11, pp.2762–2772.

9 Touihri, L., Ahmed, S.B., Chtourou, Y., Daoud, R. and Bahloul, C. (2012) 'Design of different strategies of multivalent DNA-based vaccination against rabies and canine distemper in mice and dogs', *Virol. J*., Vol. 9, No. 11, pp.319–1–10.

10 Galdiero, S., Falanga, A., Vitiello, M., Cantisani, M., Marra, V. and Galdiero, M. (2011) 'Silver nanoparticles as potential antiviral agents', *Molecules*, Vol. 16, No. 10, pp.8894–8918.

11 Elechiguerra, J.L., Burt, J.L., Morones, J.R., Camacho-Bragado, A., Gao, X., Lara, H.H. and Yacaman, M.J. (2005) 'Interaction of silver nanoparticles with HIV-1', *J. Nanobiotechnol*., Vol. 3, pp.6-1–10.

12 Lara, H.H., Ayala-Nuñez, N.V., Ixtepan-Turrent, L. and Rodriguez-Padilla, C. (2010) 'Mode of antiviral action of silver nanoparticles against HIV-1', *J. Nanobiotechnol*., Vol. 8, pp.1-1–10.

13 Lu, L., Sun, R.W., Chen, R., Hui, C.K., Ho, C.M., Luk, J.M., Lau, G.K. and Che, C.M. (2008) 'Silver nanoparticles inhibit hepatitis B virus replication', *Antiviral Ther*., Vol. 13, No. 2, pp.253–262.

14 Baram-Pinto, D., Shukla, S., Perkas, N., Gedanken, A. and Sarid, R. (2009) 'Inhibition of herpes simplex virus type 1 infection by silver nanoparticles capped with mercaptoethane sulfonate', *Bioconjugate Chem*., Vol. 20, No. 8, pp.1497–1502.

15 Lara, H.H., Garza-Treviño, E.N., Ixtepan-Turrent, L. and Singh, D.K. (2011) 'Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds', *J. Nanobiotechnol*., Vol. 9, pp.30-1–8.

16 Speshock, J.L., Murdock, R.C., Braydich-Stolle, L.K., Schrand, A.M. and Hussain, S.M. (2010) 'Interaction of silver nanoparticles with Tacaribe virus', *J. Nanobiotechnol*., Vol. 8, pp.19-1–9.

17 Mehrbod, P., Motamed, N., Tabatabaian, M., Soleimani, E.R., Amini, E. and Shahidi, M. (2009) 'In vitro antiviral effect of nanosilver on influenza virus', *Daru,* Vol. 17, No. 2, pp.88–93.

18 von Messling, V., Zimmer, G., Herrler, G., Haas, L. and Cattaneo, R. (2001) 'The hemagglutinin of canine distemper virus determines tropism and cytopathogenicity', *J. Virol*., Vol. 75, No. 14, pp.6418–27.

19 Lamb, R.A., Paterson, R.G. and Jardetzky, T.S. (2006) 'Paramyxovirus membrane fusion: lessons from the F and HN atomic structures', *Virology*, Vol. 344, No. 1, pp.30–37.

20 Blixenkrone-Møller, M., Pedersen, I.R., Appel, M.J. and Griot, C. (1991) 'Detection of IgM antibodies against canine distemper virus in dog and mink sera employing enzyme-linked immunosorbent assay (ELISA)', *J. Vet. Diagn. Invest*., Vol. 3, No. 1, pp.3–9.

21 von Messling, V., Harder, T.C., Moennig, V., Rautenberg, P., Nolte, I. and Haas, L. (1999) 'Rapid and sensitive detection of immunoglobulin M (IgM) and IgG antibodies against canine distemper virus by a new recombinant nucleocapsid protein-based enzyme-linked immunosorbent assay', *J. Clin. Microbiol*., Vol. 37, No. 4, pp.1049–1056.

22 Sharma, H.S., Hussain, S., Schlager, J., Ali, S.F. and Sharma, A. (2010) 'Influence of nanoparticles on blood-brain barrier permeability and brain edema formation in rats', *Acta Neurochir. Suppl*., Vol. 106, pp.359–364.