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ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 12, Issue, 03, pp.11581-11585, March, 2021

RESEARCH ARTICLE

ANALYSIS OF RISK FACTORS ASSOCIATED WITH CANINE PARVOVIRUS INFECTION IN VACCINATED DOGS

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ARTICLE INFO

Article History:
Received 18th December, 2020
Received in revised form
20th January, 2021
Accepted 14th February, 2021
Published online 30th March, 2021

Key words:
Canine Parvovirus, Dogs, Risk factors, Vaccination, Nested PCR.

ABSTRACT

Canine Parvovirus-2 (CPV-2) causes hemorrhagic gastroenteritis in dogs. It spreads rapidly in dog population. Three major antigenic variants distributed among the dog population worldwide at present are 2a, 2b and 2c. There are several reports implicating CPV for the causation of disease both in vaccinated and unvaccinated canine population from different parts of the country. In the present study, various risk factors implicated in the occurrence of disease particularly in vaccinated dogs were studied. Various risk factors viz. season, age, sex, breed of dog, financial status of owner, vaccination status and severity of clinical signs were analyzed in the dogs positive for CPV by Nested PCR using Fisher's exact test. Upon analysis it was concluded from the study that the risk factors included in the study were insignificantly different for the occurrence of CPV infection in vaccinated dogs and hence could not be attributed to the cause of vaccination failure in dogs for CPV.

Citation: ***Sankalp Singh Kushwaha, Gurpreet Kaur, Mudit Chandra, Neeraj Kashyap and P N Dwivedi.*, 2021.** "Analysis of risk factors associated with canine parvovirus infection in vaccinated dogs", *Asian Journal of Science and Technology*, 12, (03), 11581-11585.

INTRODUCTION

Canine Parvovirus gastroenteritis in dogs is caused by single stranded DNA non-enveloped icosahedral virus with an approximate diameter of 20 nm belonging to the genus *Parvovirus*[1]. Virus survives in the environment leading to longer persistence in kennels and shelters due to its physicochemical properties. Phylogenetic analysis revealed that CPV originated from feline panleukopenia virus (FPLV) or a very closely related carnivore parvovirus of feral canids like fox and mink[2]. It is most common in puppies of 6-20 weeks of age just when the maternal antibody protection wanes off and vaccination inadequately/insufficiently protects puppies against the infection[3, 4]. In the late 1970s and early 1980s, both live and inactivated FPLV vaccines were used to protect dogs against CPV due to the presence of shared antigens that stimulated cross protection. However, level of protection was poor and the duration of the immunity was shorter. Thus, these vaccines were replaced by killed and attenuated CPV vaccines which provided excellent protection and longer immunity[5]. As of today attenuated canine parvovirus type 2b or the original CPV type 2 is being used in the vaccines commercially.

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Despite these vaccines, still there are reported cases of CPV infection (CPV type 2) in dogs even after vaccination. In a study Truyen[6] raised concerns that vaccines used currently to prevent CPV infections in adult may fail to effectively protect pups against CPV type 2 antigenic variants. In another study CPV was reported in pups born to vaccinated bitches[7] raising concerns to update current vaccines by replacing the original CPV2 with currently circulating CPV2 variants in local canine population. Besides immediately thinking of replacing current vaccines various risk factors viz. season, age, sex, breed of dog and financial status of owner could be studied in order to identify its significant role in the survival and persistence of the virus in the environment leading to vaccination failure. As vaccine success is also dependent on the host as well as its environment, thus the present study was envisaged to study various risk factors that might contribute to occurrence of CPV in vaccinated dogs with a view to identify one or more factors that could contribute in the causation of disease.

MATERIALS AND METHODS

Preparation of questionnaire for analysis of risk factors : A questionnaire was prepared to study various factors relating to the causation of CPV in dogs. These included season, age, sex and breed of dogs, vaccination status of dogs, economic status of the owner and deworming status of the animal. The

questionnaire was filled before the collection of samples from the owners individually.

***Ethical permission*:** The institutional animal ethics committee permission was obtained vide GADVASU/2013/IAEC/ 18/LA015.

***Collection of samples*:** Samples were collected from the veterinary clinics in Ludhiana, Punjab (n=89) and the veterinary hospitals in Bhopal, Madhya Pradesh (n=11). Samples were collected from February 2017 to June 2018. Rectal swabs (n=100) were collected in phosphate buffer saline (pH 7.2) from dogs exhibiting clinical signs of gastroenteritis, hemorrhagic enteritis and pyrexia. All the rectal swabs were kept in 4°C till further processing.

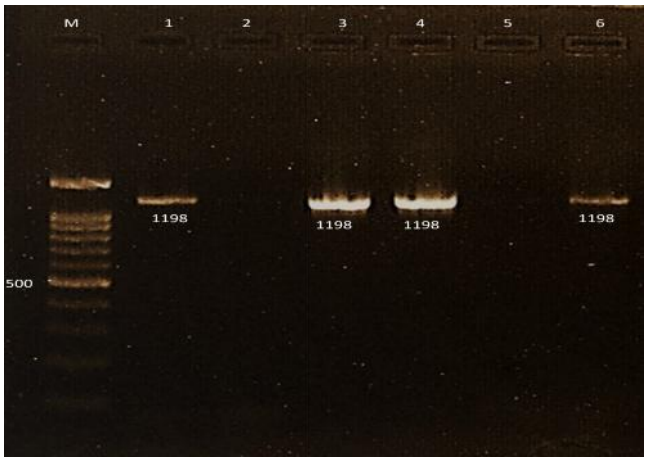
Extraction of DNA: The DNA was extracted from all the samples (rectal swabs) and a vaccine (Nobivac DHPPi, Intervet Pvt. Ltd.) using phenol-chloroform extraction method as described by Sambrook and Russell[8]. Vaccine was used as a positive control and nuclease free water was used as a negative control throughout the study.

Polymerase Chain Reaction (PCR) and Nested PCR (NPCR) for the detection of CPV: The primers used in the study for PCR and NPCR were as per Mizak and Rzezutka[9]. The PCR reaction was set up by adding 5.0 µl of 10X PCR buffer (with 15mM $MgCl_2$), 1.0 µl each of forward (5'-AGCTATGAGATCTGAGACAT-3') and reverse primer (5'-AGTATGTTAATATAATTTTCTAGGTGC-3') (25 pm/µl), 1.0 µl of dNTPs mix (10 mM), 1 U Taq DNA polymerase, 15 µl of the template DNA and final volume was made upto 50 µl using nuclease free water. NPCR reaction was set up by adding 5 µl of PCR product from above reaction, 2.5 µl of 10X PCR buffer (with 15mM $MgCl_2$), 1.0 µl each of forward (5'-ATACAGGAAGATATCCAGAAG-3') and reverse primer (5'-AGTATGTTAATATAATTTTCTAGGTGC-3') (25 pm/µl), 1.0 µl of dNTPs (10 mM), 1 U Taq DNA polymerase and volume was made upto 25µl by adding nuclease free water. The cycling conditions for the PCR and NPCR were as follows; 35 cycles of denaturation at 94°C for 60S, annealing at 55°C for 60S, elongation at 72°C for 150S and a final elongation at 72°C for 10 min in a thermocycler (Veriti, Life Technologies, USA).

Statistical Analysis: The results of NPCR were compared with various risk factors included in the questionnaire using Fisher's exact test.

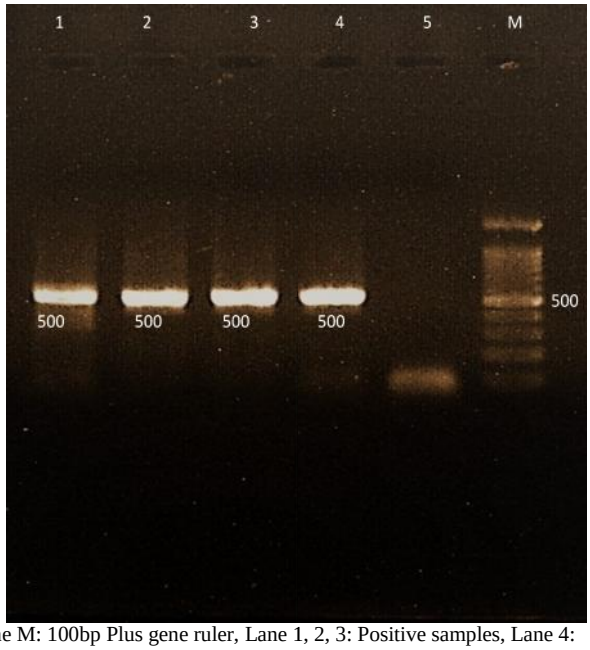
RESULTS

PCR and NPCR for the detection of CPV : PCR is a simple, sensitive and specific method for the detection of CPV directly from faeces of infected dogs. The DNA extracted from the rectal swab was subjected to PCR and revealed that out of a total of 100 samples, 18 were found positive yielding a product size of 1198 bp (Fig 1). Out of these 18 positive, seven were from dogs with a vaccination history. All the 100 PCR products when subjected to NPCR revealed 63 samples to be positive by NPCR (Fig 2). Out of these 63 positive, 30 were from dogs with a vaccination history. Also out of 63 positive six were from Bhopal, Madhya Pradesh (6/11) and 57 were from Ludhiana, Punjab (57/89).



Lane M: 100bp Plus gene ruler, Lane 1: Positive control, Lane 2: Negative control, Lane 3, 4 and 6: Positive samples, Lane 5: Negative sample.

Fig. 1. PCR for detection of CPV



Lane M: 100bp Plus gene ruler, Lane 1, 2, 3: Positive samples, Lane 4: Positive control, Lane 5: Negative control.

Fig. 2. Nested PCR for detection of CPV

Statistical analysis of various risk factors leading to cause of CPV in dogs : The results for each risk factor were analyzed in two groups. One group included total number of samples (n=100) and the other group included samples from dogs that had vaccination history (n=51). The results of NPCR were compared with various risk factors included in the questionnaire using Fisher's exact test.

***Analysis of risk factor 'Season'* :** Out of the 100 samples most of the cases were observed during summer season (n=50) followed by rainy (n=37) and winter season (n=13). Out of the samples collected during summer season 80% (40/50) were positive for CPV and out of samples collected during winter season 76.9% (10/13) were positive for CPV whereas none of the sample (0/37) was positive for CPV in rainy season (Table 1) ($P \leq 0.001$). The results suggest significant variation in the prevalence of CPV when compared during different seasons indicating that seasons have a role in the transmission or survival of CPV. Out of the 51 samples from vaccinated dogs most of the cases 73.9% occurred during summer (17/23) followed by 66.67% in winters (6/9) and 36.8% in rainy

Table 1. Risk factors analysis from samples collected from clinically CPV positive dogs

Risk Factors		Total samples (n=100)		P Value (≤)
		NPCR Negative	NPCR Positive	
Season of sample collection	Rainy	24	13	0.001
	Summer	10	40	
	Winter	3	10	
Sex of dog	Male	27	31	0.0226
	Female	10	32	
Age of dog	≤ 6 months	31	48	0.4510
	> 6 months	6	15	
Breed of dog	Small	5	6	0.2002
	Medium	12	32	
	Large	20	25	
Financial status of owner	Below 5 lakhs	28	50	0.8031
	More than 5 lakhs	9	13	
Intensity of diarrhea	Diarrhea	13	15	0.2538
	Bloody diarrhea	24	48	
Vaccination and booster status	No	16	33	0.4130
	Yes	21	30	
Deworming status	No	14	22	0.8306
	Yes	23	41	

Table 2. Risk factor analysis from clinically CPV positive dogs with vaccination history

Risk Factors		Total samples (vaccinated dogs) (n=51)		P Value (≤)
		NPCR Negative (Per cent)	NPCR Positive (Per cent)	
Season of sample collection	Rainy	12 (23.52)	7 (13.72)	0.0439
	Summer	6 (11.76)	17 (33.33)	
	Winter	3 (5.88)	6 (11.76)	
Sex of dog	Male	16 (31.37)	16 (31.37)	0.1426
	Female	5 (9.80)	14 (27.45)	
Age of dog	≤ 6 months	17 (33.33)	22 (43.13)	0.7391
	> 6 months	4 (7.84)	8 (15.68)	
Breed of dog	Small	2 (3.92)	3 (5.88)	0.9139
	Medium	7 (13.72)	12 (23.52)	
	Large	12 (23.52)	15 (29.41)	
Financial status of owner	Below 5 lakhs	15 (29.41)	23 (45.09)	0.7499
	More than 5 lakhs	6 (11.76)	7 (13.72)	
Intensity of diarrhea	Diarrhea	6 (11.76)	8 (15.68)	1.0000
	Bloody diarrhea	15 (29.41)	22 (43.13)	
Vaccination and booster status	No	8 (15.68)	6 (11.76)	0.2069
	Yes	13 (25.49)	24 (47.05)	
Deworming status	No	4 (7.84)	6 (11.76)	1.0000
	Yes	17 (33.33)	24 (47.05)	

season (7/19) (Table 2) (P< 0.0439). The results suggests that among vaccinated dogs seasons had no affect and do not contribute to disease.

Analysis of risk factor 'sex of the dog': Out of the 100 samples collected from dogs 58% of the cases were in males (n=58) and 42% among females (n=42). However, more positive cases 53.44% were observed in females (32/42) compared to 51.72 % in males (31/58) as positive for CPV by NPCR (Table 1) (P≤ 0.0226). Out of the 51 samples collected from vaccinated dogs 73.68% positive cases were observed in female dogs (14/19) as compared to 50% in male dogs (16/32) (Table 2) (P≤ 0.1426). The results suggest no significant variation in the prevalence of CPV when compared among sexes.

Analysis of risk factor 'age of dog': Out of the 100 samples collected from dogs 79% cases were observed in the age group below 6 months (n=79) and only 21% cases were in dogs having age above 6 months (n=21). Out of the samples collected from dogs having age below 6 months 60.75% were positive for CPV (48/79) and 71.42% were positive in dogs having age above 6 months (15/21) by NPCR (Table 1) (P≤ 0.4510). Out of the 51 samples collected from vaccinated dogs 56.41% were observed in the age below 6 months (22/39) and 66.66% were observed in the dogs above 6 months (8/12) (Table 2) (P≤ 0.7391). The results suggest no significant variation in the prevalence of CPV when compared among different age groups.

Analysis of risk factor 'breed of dog' : Out of the 100 samples collected from dogs 45% were observed in large breed size dogs (n=45), 44% in the medium breed size dogs (n=44) and 11% in the small breed size dogs (n=11) (Table 1). Out of the samples collected from dogs of large size breeds 55.56% (25/45,) were positive for CPV, 72.73% were positive from medium size breeds (32/44,) and none from small size breed dogs (P≤0.2002). Large breed size dogs included Labrador, German shepherd, and Rottweiler. Medium size dogs included non-descript breeds of dogs. Out of the 51 samples collected from vaccinated dogs 63.16% positive cases were observed in medium breed size dogs (12/19) followed by 55.56% in the large breed size dogs (15/27) (Table 2) (P≤0.9139). The results suggest no significant variation in the prevalence of CPV when compared among different breeds of dogs.

***Analysis of risk factor 'financial status of the owner'*:** Out of the 100 samples collected from dogs most of the cases 78% were observed in owners having financial status below 5 lakhs (n=78) and only 22% among owners having financial status

above 5 lakhs (n=22) (Table 1). Out of the samples collected from the dogs of the owner having financial status below five lakhs per year, 64.10% were positive for CPV by NPCR (50/78) (P≤ 0.8031). Out of the 51 samples collected from vaccinated dogs 60.52% of the positive cases were observed in owners having financial status below 5 lakhs (23/38) and 53.84% among owners having financial status above 5 lakhs (7/13) (Table 2) (P≤0.7499). The results suggest no significant variation in the prevalence of CPV when compared among different financial status of the owner indicating that disease is affected both rich and poor equally.

Analysis of risk factor 'intensity of diarrhea': Out of the 100 samples collected from dogs 72% cases were observed in dogs exhibiting haemorrhagic diarrhea (n=72) followed by 28% dogs exhibiting only diarrhea (n=28) (Table 1). Out of the samples collected from dogs exhibiting haemorrhagic diarrhoea, 66.66% were positive for CPV (48/72) and 53.57% were positive in dogs exhibiting only diarrhea (15/28) (P≤ 0.2538). Out of the 51 samples collected from vaccinated dogs 59.45% cases were observed in dogs exhibiting haemorrhagic diarrhoea (22/37) followed 57% in the dogs exhibiting only diarrhoea (8/14) (Table 2) (P≤ 1.0).

Analysis of risk factor 'vaccination status' : Out of the 100 samples collected 67.35% positive were from unvaccinated dogs (33/49) and 58.82% positive were from dogs which had been vaccinated for CPV (30/51) (Table 1) (P≤ 0.4130). Out of the 51 samples collected from vaccinated dogs, 42.86% dogs were positive for CPV which had first dose of vaccine (6/14) and 64.86% in the dogs which had received booster for CPV (24/37) (Table 2) (P≤ 0.2069).

Analysis of risk factor 'deworming status': Out of the total samples collected in both the groups (total sample group and the vaccinated group) cases were almost similarly affected if the dogs were dewormed at the right stage with the right time interval between deworming and vaccination (Table 1 and 2) (P≤ 0.8306 and P≤ 1.00).

DISCUSSION

In the present study some dogs that were positive for CPV by NPCR were vaccinated indicating that it might be possible that vaccination of pups against CPV is not conferring immunity against CPV. This might be due to the mismatching of vaccine strain and the CPV strain causing infection in dogs. Similar observation of occurrence of CPV in vaccinated dogs has been observed in earlier studies too[10]. In the present study majority of CPV cases were observed during summer followed by winter and rainy season and the same was observed in vaccinated dogs. This could be attributed to the intense and prolonged summer as well as winter being faced in Northern part of the India leading to greater stress on the animals making them prone to infection. No effect of sex or breed was observed in the study similar to the findings of the earlier studies indicating that all sexes and breeds of dogs were equally susceptible to CPV-2 infection[11, 12]. In the present study, most of the animals affected were below 6 months of age substantiating already established fact that the infection caused by CPV is more severe in younger animals[13, 14, 15, 16, 17]. An investigation into ages of dogs prone to canine parvovirus infection in Brazil showed that infection occurred mostly in 2-4 months old puppies[11, 18]. In Slovenia, 67.6% of deaths due to CPV infection was observed in dogs below six months of age, followed by 25.7% in dogs aged between six months to one year, 6.8% in dogs one year old and above[12] indicating younger dogs more susceptible to CPV. Tilley and Smith[19] too stated that many cases of CPV infection in dogs are seen between six weeks and six months of age with the disease being more severe in younger puppies. Also, the above observations relating to sex of the dog were in tandem to observed reports of no significant difference among the sexes of dogs with CPV infection in Rio de Janeiro[11]. However there were also reports that the prevalence of CPV infection was higher in males when compared to females[20, 21, 22]. Breed wise comparison indicated that Labrador and German shepherd breeds of dogs were mostly affected by CPV as detected by NPCR. These observations too were in tandem to the earlier reported facts in which Kumar et al[23] and Singh et al[17] stated that in India German Shepherd, Labrador and Pomeranian breeds of dogs are most predisposed for CPV. It has also been reported that Doberman pinscher, Rottweiler and German shepherd dogs appear to be under greater risk of developing parvo viral enteritis[20, 24].

Local breeds of dogs were the least susceptible to the infection when compared with the foreign breeds similar to the findings of an earlier study[25]. Local breeds have a greater degree of resistance against the virus and have been regarded as healthy carriers of CPV[26]. This is of great epidemiological relevance as they play important roles in distribution of the virus indiscriminately to other breeds due to their free ranging habits[25]. Local breeds pose a great danger to the foreign breeds of dogs that are more susceptible to the agent[26]. Thus, upon analysis of various risk factors viz. season, age, sex, breed of dog, financial status of owner, vaccination status and severity of clinical signs it could be concluded that these risk factors included in the study were insignificant in differentiating vaccinated and non vaccinated dogs. However, further studies incorporating still more factors with an increased sample size could shed more light on this in vaccinated dogs.

Conclusion

It could be concluded from the study that CPV positive cases were observed in vaccinated dogs suspected for CPV using polymerase and nested polymerase chain reaction. The risk factors selected in the study when statistically analysed could not be attributed to the occurrence of disease in vaccinated dogs indicating more factors to be studied to establish if there is any correlation between vaccination failure and the risk factors.

Acknowledgements

The authors are thankful to DBT for funding the project BT/ADV/Canine Health/TANUVAS/2017-18 and to the Director of Research, Guru Angad Dev Veterinary and Animal Sciences University for the support.

Conflicts of interest: The authors declare that they have no conflicts of interest.

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