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Evaluation of maternal antibodies against parvovirus in puppies with vaccinated and unvaccinated bitches in Mazandaran Province, Iran

[*Avaliação de anticorpos maternos contra o parvovírus em filhotes de cadelas vacinadas e não vacinadas na província de Mazandaran, Irã*]

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ABSTRACT

Canine parvovirus 2 (CPV-2) is a contagious high-risk virus in dogs, which emerged as an important pathogen in 1978. There are limited investigations that explore maternally derived antibody (MDA) in canine parvovirus in puppies around the world. Furthermore, there is no such research in any province of Iran. This study measured the serum level of MDA against parvovirus in 42 puppies (21 puppies with vaccinated bitches and 21 puppies with unvaccinated bitches) and the serum level of canine parvovirus antibodies of their bitches (n=28) (21 vaccinated and 7 unvaccinated bitches). Antibodies against parvovirus were measured using quantitative, enzyme-linked immunosorbent assay (ELISA). Our results showed that 62% (13 out of 21) of puppies from vaccinated bitches and 76% (16 out of 21) of puppies from unvaccinated bitches were positive for anti-parvovirus antibodies, which wasn't significantly different (P=0.253). Moreover, puppies' titers weren't statistically different in vaccinated and unvaccinated groups (P=0.476). There was a similar condition between vaccinated and non-vaccinated bitches (P=0.583). There was no relationship between breed and sexuality with vaccination status (Ps>0.05).

Keywords: puppies, canine parvovirus, anti-parvovirus antibodies, maternally derived antibody, Mazandaran Province

RESUMO

O parvovírus canino 2 (CPV-2) é um vírus contagioso de alto risco em cães, que surgiu como um importante patógeno em 1978. Há poucas pesquisas que exploram o anticorpo derivado da mãe (MDA) no parvovírus canino em filhotes em todo o mundo. Além disso, não há pesquisas desse tipo em nenhuma província do Irã. Este estudo mediu o nível sérico de MDA contra o parvovírus em 42 filhotes (21 filhotes com cadelas vacinadas e 21 filhotes com cadelas não vacinadas) e o nível sérico de anticorpos contra o parvovírus canino de suas cadelas (n=28) (21 vacinadas e 7 não vacinadas). Os anticorpos contra o parvovírus foram medidos por meio de um ensaio imunoenzimático (ELISA) quantitativo. Nossos resultados mostraram que 62% (13 de 21) dos filhotes de cadelas vacinadas e 76% (16 de 21) dos filhotes de cadelas não vacinadas foram positivos para anticorpos antiparvovírus, o que não foi significativamente diferente (P=0,253). Além disso, os títulos dos filhotes não foram estatisticamente diferentes nos grupos vacinados e não vacinados (P=0,476). Houve uma condição semelhante entre as cadelas vacinadas e as não vacinadas (P=0,583). Não houve relação entre a raça e a sexualidade com o status da vacinação (Ps>0,05).

Palavras-chave: filhotes, parvovírus canino, anticorpos antiparvovírus, anticorpos derivados da mãe, província de Mazandaran

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INTRODUCTION

Canine parvovirus infection is caused by canine parvovirus- type 2 (CPV-2), a member of the family Parvoviridae, Genus Parvovirus. This virus originated from parvovirus of cat or wild carnivorous species. Then, this virus evolved into the two antigenic variants –namely CPV-2a and CPV-2b, where CPV-2b emerged as the predominant pathogen in dog worldwide (Appel *et al*., 1979; Black *et al*., 1979; Carmichael, 2005; Martella *et al*., 2006; Goddard and Leisewitz, 2010). The third antigenic variation of canine parvovirus (i.e., CPV-2c) was detected in Italy in 2000 (Buonavoglia *et al*., 2001).

The parvovirus is transmitted through oral or oral-nasal contact when the animal is exposed to feces, vomits, or other contaminated material. Furthermore, this virus may spread through birds, rodents, or insects. The clinical features of this disease vary from silent form, pre-acute or sudden death. The clinical disease usually occurs in dogs younger than 6 months of age or immune-suppressed adult dogs (McCaw and Hoskins, 2006; Kalli *et al*., 2010). Usual clinical signs include vomiting, diarrhea, severe dehydration/hypovolemia, systemic inflammatory response syndrome (SIRS), hypercoagulability, endotoxemia, metabolic acidosis (or alkalosis), and death (Goddard and Leisewitz, 2010; Kalli *et al*., 2010). Wide ranges of various techniques are employed to diagnose CPV, including Latex Agglutination Test (LAT), Enzyme-Linked Immunosorbent Assay (ELISA), Haemagglutination (HA), Counter Immunoelectrophoresis (CIE), Polymerase Chain Reaction (PCR) and Restriction Enzyme (RE) digestion, real-time PCR, Electron Microscopy (EM), virus isolation using in MDCK, CRFK or A 72 cell line, Virus neutralization test, and Fluorescent Antibody Test (FAT) (Appel *et al*., 1979; Nandi and Kumar, 2010; Mylonakis *et al*., 2016).

Because of the endotheliochorial placentas in dogs, only 2-18% of maternally derived antibody (MDA) can be transferred to the fetus, which protects puppies for a short time after birth. In contrast, a high concentration of MDA in colostrum can protect puppies for a longer period, which decreases gradually during lactation. MDA has an inhibitory effect on the production of antibodies in puppies, so that immunoglobulin production begins when MDA falls below a certain level (Greene and Levy, 2012). There are some studies that explored MDA in puppies, but major goals of these investigations were evaluation of the interaction between routine vaccination and maternal antibody (MAb) titer to CPV (Gooding and Robinson, 1982; Iida *et al*., 1990; Waner *et al*., 1996; Mila *et al*., 2014). Furthermore, to the best of our knowledge, no study has evaluated the situation of maternal antibodies against canine parvovirus in Iran. Hence, we conducted this investigation to assess MDA against parvovirus in puppies from vaccinated and none vaccinated mothers in Mazandaran province, Iran.

MATERIALS AND METHODS

This study was performed during the period from August 2019 to May 2020.

The current research has been done in Mazandaran province located along the southern coast of the Caspian Sea and in the adjacent Central Alborz Mountain range, in central-northern Iran. The total area, total population, and density of this province are 23,833km^2, 3,073,943, and 130km^2, respectively. The climate of Mazandaran is divided into three types: moderate Caspian weather, moderate mountainous weather, and cold mountainous weather. Mazandaran is divided into 20 counties.

Blood samples were taken from 21 puppies and their vaccinated bitches (n=42), 21 puppies, and their unvaccinated bitches (n=28, only 7 bitches) in the first 9 days of birth and randomly from both sexes. All mothers were under two years old. Blood was collected for serology by cephalic venipuncture in the amount of 2-3mL using a sterile syringe and a 21-gauge needle. Blood samples were left at room temperature (30 minutes to 2 hours) for clothing and centrifuged at 3000 rpm for 10 minutes to separate the sera. Serum samples were labeled and kept frozen at minus 20 °C throughout the study period. All characteristics of puppies tested, including breed, age, sex, owner name, address, sampling date, were recorded in numbered sheets.

Anti-parvovirus antibody titer for bitches and puppies was measured with a quantitative enzyme-linked immunosorbent assay (ELISA): CPV (Canine Parvo Virus) IgG ELISA from

Demeditec (REF, DE 2475), Germany. The kit used contained both negative and positive controls, and the procedure was followed as the manufacturer recommended. The results (optical density-OD) were read using an ELISA reader (Lab system-Finland-series: 3520900102) at wavelength 450-620nm.

Data analysis was performed using SPSS.16 statistical package (Chicago, USA), and a P-value below 0.05 was considered significant. The frequency of the data was described as mean ± SD values for continuous variables and as proportions for categorical data. Antibody titer between puppies and bitches in both vaccinated and unvaccinated groups were compared using Student's t-test. An antibody titer in curve more than 1/100 was considered as the protective level, and the percentage of immunized dogs in each group was calculated and compared using Fisher's exact test. Furthermore, the correlation between breed and sexuality variables with test results was calculated using Pearson Chi-Square and Fisher's exact test.

RESULTS

Our cases included 42 puppies (21 puppies from vaccinated bitches and 21 puppies from unvaccinated bitches). Puppies were selected from different breeds, including German Shepherd, Husky, Pitbull, Pointer, Rottweiler, Terrier, and Dobermann. Puppies in the vaccinated group included 8 (38%) females and 13 (62%) males, and puppies in the unvaccinated group include 14 (66%) females and 7 (34%) males (Table 1). Our analysis using Pearson Chi-Square and Fisher's exact test indicated no relationship between breed and sexuality with vaccination status (Ps>0.05).

Table 1. Categorical information on 42 puppies in both vaccinated and non-vaccinated groups

		Vaccinated	Non-vaccinated	Total
Breed	German Shepherd	7	16	23
	Husky	4	1	5
	Pitbull	3	1	4
	Pointer	3	1	4
	Rottweiler	1	0	1
	Terrier	3	1	4
	Dobermann	0	1	1
Sex	Female	8	14	22
	Male	13	7	20

The mean age of puppies was 8.4±0.9 days, which was not significantly different between vaccinated and non-vaccinated groups in both species (P=0.415). Furthermore, the mean age of bitches was 21.6±2.7 months, which was also not statistically different between vaccinated and non-vaccinated groups (P=0.846) (Table 2).

Our findings revealed that 62% (13 out of 21) of puppies from vaccinated bitches were positive for anti-parvovirus antibody, while test results for 16 puppies from non-vaccinated bitches (76%) were positive (P=0.253). Moreover, puppies' titer was not significantly different in the vaccinated and non-vaccinated groups (P=0.476). There was a similar condition between vaccinated and non-vaccinated bitches (P=0.583) (Table 1 and 2), where 43% (9 out of 21) of vaccinated bitches and 71% (5 out of 7) of unvaccinated bitches were positive for anti-parvovirus antibody (P=0.192) (Table 2 and 3).

Table 2. Information of continuous variables on 42 puppies and their corresponding bitches in both vaccinated and non-vaccinated groups

		No.	Mean	Std. Deviation	Std. Error	Minimum	Maximum	*P* value
Bitch age (months)	Vaccinated	21	21.6	2.6	0.6	18	24	0.846
	Non-vaccinated	7	21.7	2.8	0.6	16	24	
	Total	28	21.6	2.7	0.4	16	24	
Puppy age (days)	Vaccinated	21	8.2	0.9	0.2	7	9	0.415
	Non-vaccinated	21	8.5	0.9	0.2	6	9	
	Total	42	8.4	0.9	0.1	6	9	
Bitch titer	Vaccinated	21	1.2	0.2	0.0	1.1	1.78	0.583
	Non-vaccinated	7	1.2	0.1	0.0	1.1	1.28	
	Total	28	1.2	0.2	0.0	1.1	1.78	
Puppies' titer	Vaccinated	21	1.2	0.2	0.0	1.1	1.66	0.476
	Non-vaccinated	21	1.3	0.2	0.0	1.1	1.9	
	Total	42	1.3	0.2	0.0	1.1	1.9	

Table 3. Anti-parvovirus antibody test results in puppies and their corresponding bitches in both vaccinated and non-vaccinated groups

		Vaccinated	Unvaccinated	Fisher's exact test
Bitches	Negative	12	2	0.192
	Positive	9	5	
	Total	21	7	
Puppies	Negative	8	5	0.253
	Positive	13	16	
	Total	21	21	

DISCUSSION

The current study was conducted to investigate the frequency of anti-parvovirus antibodies in puppies with vaccinated and unvaccinated bitches in Mazandaran province, Iran. Generally, our findings revealed that MDA was transferred to the puppies from both vaccinated and unvaccinated mothers. Our literature of review indicated that major of previous studies intended to investigate the interaction between MDA and subsequent vaccinations in puppies (Gooding and Robinson, 1982; Iida *et al*., 1990; Buonavoglia *et al*., 1994; Waner *et al*., 1996; Mila *et al*., 2014). Furthermore, contrary to our study, they mainly employed puppies with vaccinated bitches, while we also explored puppies with unvaccinated bitches.

For example, in a study by Gooding and Robinson (1982), the MDA titer to CPV was explored in 39 puppies in 7 litters, vaccinated with inactivated CPV. Transfer of MAb was demonstrated in 71% (5/7) of the litters. Cases with MDA titers of greater than 20 and lower than 20 showed negative and positive responses to the vaccination of puppies (Gooding and Robinson, 1982). In another study, MDA against

canine distemper virus (CDV), canine parvovirus (CPV), and infectious canine hepatitis virus (ICHV) was evaluated in 6 Beagle dams and their 38 puppies. The results indicated a positive correlation between CPV titers between puppies and dams (r= 0.793). In addition, 67% of puppies from vaccinated bitches have positive maternal antibodies with mean half-lives of 13.5 days. Again, this study confirmed that a lower maternal antibody titer (less 1:5) could stimulate a stronger antibody response against CPV vaccination in puppies (Iida *et al*., 1990). Similarly, antibody titers against CPV in ten pure-bred, pregnant Beagle dogs and 16 corresponding puppies (4 puppies for each bitch) were measured. The mean serum CPV antibody titer for the bitches was 1:320. Moreover, the mean half-lives of maternal CPV antibodies were 11.6 ± 2.5 days (Waner *et al*., 1996). Assessment of MDA on 79 puppies until 56 days of age revealed that 43% and 57% of puppies had MDA ≤1:160 and greater titers, respectively. The findings confirmed those breed size and growth rates were positively correlated with maternal antibody concentration (Mila *et al*., 2014). Intranasal vaccination using a modified live canine parvovirus vaccine could overcome MDA, where 17.6%, 72.7%, and 100% of puppies with haemagglutination inhibition antibody titer of 160, 80, and 40, respectively showed positive vaccination titer (Buonavoglia *et al*., 1994).

Our study can be considered as a starting point to run further research to explore MDA against canine parvovirus in more detail. Investigation on a larger population of puppies and the corresponding parents and measures of MDA at different intervals (such as different ages and various vaccination steps) could result in more accurate and reliable findings.

CONCLUSIONS

According to the results of this study, it can be concluded that although vaccination of the bitches against canine parvovirus for most puppies causes higher serum IgG levels than puppies with non-vaccinated mothers, this level of IgG cannot protect all of them from the canine parvovirus. Therefore, caring for puppies until the first period of vaccination is necessary and inevitable.

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