



TWO-SHOTS OF IMMUNIZATION EFFECTIVELY INDUCE PROTECTIVE IGG AGAINST CANINE PARVO-VIRAL INFECTION

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ABSTRACT

Regardless of good hygiene and management, a significant number of dogs are lost every year due to deadly attack of parvo virus. The immunization schedules recommended by the World Small Animal Veterinary Association (WSAVA) do not have exclusive suggestions on dogs living in Bangladesh. The WSAVA Vaccination Guidelines Group (VGG) suggested that vaccination recommendations that apply to a developed country may not be appropriate for a developing country. Due to inadequate sero-monitoring, emergence and re-emergence of Parvo viral infection following immunization is a common event. Therefore, the current study is designed to establish a definite guideline for Parvo viral immunization in dogs. We immunized group of dogs with commercially available parvo virus vaccine Nobivac Dog®, and a second dose was injected 14-days following the initial dose. Sera samples were collected before starting injections and 7-day following 1st and 2nd dose of vaccinations. We performed Enzyme Linked Immunosorbent Assay (ELISA) to detect protective serum IgG titer against the canine parvo virus type 2 antigen. Demographic data of the participated animals was compared to the status of antibody titer. Findings of this study revealed that two-shots of immunizations with a booster injection of 2-3 weeks apart from the primary immunization is essential to develop positive titer. The purebred female dogs of older than three-months had higher IgG when compared to local, crossbred, male and younger dogs. The results will provide valuable information to establish National Guidelines for Immunizations in Dogs of Bangladesh for effective prevention and control of this deadly disease.

Contribution/Originality: Sero-monitoring of the canine anti-parvo virus IgG is not a common practice in Bangladesh following vaccination. Every year a significant number of dogs are lost owing to this deadly virus due to improper vaccination. The current study incorporated essential data regarding parvo virus vaccination to the veterinarians and dog-owners.

1. INTRODUCTION

Dogs are kept by the people to relieve their loneliness and accompany during leisure or as part of enjoyment. However, some people keep them as a faithful guard. In Bangladesh, total population of dog is more than two million [1]. As the dogs have a close companionship with the human, it is very important to keep them sound and

healthy, and the dog owners usually do so with their best efforts. Even though, dogs are affected by a reported number of diseases every year [2-4]. A study on pet dogs in Dhaka, Bangladesh reported higher prevalence rates of rabies, canine distemper, liver disease, nephritis, bronchitis and pneumonia, diarrhea, and other metabolic, infectious and non-infectious diseases [5]. Another recent study on the same population reported similar disease prevalence with the highest number of dermatological cases followed by canine parvo viral infections [2]. Costs of medication, extra care during treatment or sometimes death and waste of working hour of the pet owners cause significant economic loss every year.

Intimate relationship of dogs with human being may sometimes arises major public health issues warning the One Health as the animal share same environment with humans and harbor a noticeable number of infectious bugs transmissible to human and other food animals [6-13]. Home pets were reported to play a distinct direct role in transmitting zoonotic diseases [14]. Zoonotic diseases are very common everywhere in the world. It is estimated that more than 60% of infectious diseases and 30% of new or emerging infectious diseases in people are transmitted from animals [15]. According to the World Health Organization (WHO), dogs and cats are responsible for more than 96% of human rabies cases in South-East Asia [16].

To keep the dogs healthy and sound, and to prevent infections, routine deworming and pre-immunization with vaccines are recommended by the Vaccination Guideline Group (VGG) of the World Small Animal Veterinary Association (WSAVA) and other veterinary associations [17-20]. Moreover, different countries have their own National Immunization Schedules for dogs and cats based on local or regional prevalence and incidence of diseases [21-23].

Bangladesh Veterinary Association (BVA) or Department of Livestock Services (DLS) of Bangladesh does not have National Immunization Guidelines for pet dogs and cats. Bangladeshi veterinarians and pet owners follow different immunization schedules according to their own experiences or sometimes follow different country's guidelines. Most veterinarians suggest booster immunization following one year of primary immunization for Parvo virus although World Health Organization (WHO) suggests first boost after six months followed by yearly booster [16]. Moreover, there is no routine sero-survey on the protective antibody titer monitoring following immunizations. Hence, the events of immunization failure, particularly for canine parvo viral infections are a common affair faced by the veterinarians issuing a huge economic loss due to expensive medications and loss of pet dogs. Therefore, it is a current demand to take effective preventive and control measures against Parvo virus infection of dogs in Bangladesh.

2. MATERIAL AND METHODS

2.1. Study Area, Duration, and Population

Dogs visited SAQ Teaching Veterinary Hospital (SAQTVH) of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram during the period of September 2020 to June 2021 were included in the current study. Tests and data analysis were performed during July-August 2021.

2.2. Ethics Approval

The research has been conducted in accordance with the institutional guidelines according to the requirements of the CVASU Animal Ethics Committee. Animal Ethics approval has been taken, approval no. CVASU/Dir 'Research & Extension' (R&E) 'Ethics Committee' EC/2020/165(5). The handling, clinical examination and sample collection from the animals were executed according to the current Bangladesh legislation Cruelty to Animals Act 1920 (Act No. 1).

2.3. Immunization of Dogs

A total of 40 clinically healthy puppies of 45-90 days old were categorized into i) experimental (n=20), and ii) control group (n=20). The experimental dogs were immunized with commercially available combined dog vaccine having parvo virus vaccine (Nobivac Dog®) and were injected sub-cutaneously at day 0 and 14 following standard procedures of injections (Figure 1a). The control groups were injected with distilled water or 0.9% NaCl normal saline (NS®).

2.4. Data and Sample Collection

Demographic data of the dogs including gender, breed, age, companionship, source, previous or current history of illness were collected using a standard questionnaire. Baseline serum samples were collected from all animals before starting experiments. For detection and analysis of anti-parvo virus antibody titer, blood samples from each animal were collected at day 7 and 21 after primary injection and under physical restraint. Sera were separated by placing the blood sample undisturbed for 30 minutes followed by spinning for 10 minutes at 1500 rpm. The samples were stored at -20⁰ C until ELISA performed.

2.5. Serum Antibody Detection by ELISA

Serum IgG against canine Parvo virus was detected using commercially available canine Parvo virus type 2 antigen coated ELISA kits (cat#ARG80990, Arigo Biolaboratories Co.). The procedure of ELISA described by Salvi, et al. [24] was followed with modifications when necessary. Briefly, purified inactivated Canine Parvo Virus pre-coated microtiter plate was supplied in the kit. Using sample diluent, the sera samples were diluted to 1:250 and 100uL was put in duplicates. Positive control, negative control and blank (wash buffer) were placed in appropriate wells. The plate was incubated for 1h at 37°C. Following wash with wash buffer, 100uL of horse-reddish peroxidase (HRP)-conjugated secondary antibody was added and incubated for 1h at 37°C. Following wash, equal volume of substrate A and B was mixed and 100µL was added for 30 minutes at room temperature. Stop solution (50µL) was added to pause the further color development. Optical density (OD) was measured immediately using an automated microtiter plate reader at 450nm wavelengths.

2.6. Statistical Analysis

The OD values from the control and vaccinated dogs was distributed normally and hence the comparison was tested using t test in GraphPad Prism 7.0 software. The comparison between the demographic parameters and anti-parvo virus OD values was performed using unpaired t test or ANOVA. The statistical analysis performed is provided in the figure legends. A p value of ≤0.05 was considered significant.

3. RESULTS

3.1. Demographic Data of the Experimental Population

The current study population revealed that the majority number of dogs were local or non-descriptive male and mostly adopted from someone else Table 1. Most dogs received vaccines at their early life of less than three-month age indicating that the owners of the dogs are aware about the vaccination. Findings of the current study also revealed that people keep dogs as their companion rather than just a pet.

3.2. Antibody Analysis of Dogs Following Canine Parvo Virus Vaccination

The current study demonstrated that the sera from dogs with no history of vaccination against canine parvo virus type 2 had low OD values and this finding is like the negative control of the ELISA kit Figure 1b. On the other hand, the sera from dogs received single dose of Parvo virus vaccine showed significantly ($p<0.05$) higher OD values compared to the OD values of sera from unvaccinated dogs. This OD values however, lower than the positive

control that indicates the protective level of the antibodies in the vaccine recipients. Therefore, we can suggest that single dose of Parvo virus vaccine provides only protection nearly to the borderline. However, the sera from dogs received two-shots of vaccines showed the maximum generation of antibodies indicated by the highest OD values ($p < 0.01$) and exceeds the protective level.

Table 1. Demographic characteristics of dogs under study.

Variables	Categories	n =	% of total
Sex of dogs	Male	26	65
	Female	14	35
Breed	Purebred	14	35
	Crossbred	4	10
	Non-descriptive/local	22	55
Source of dogs	Adopted	28	70
	Pet shop	12	30
Age	<3 months	25	62.5
	>3 months	15	37.5
Companionship	Companion	25	62.5
	Pet	13	32.5
	Stray	2	5

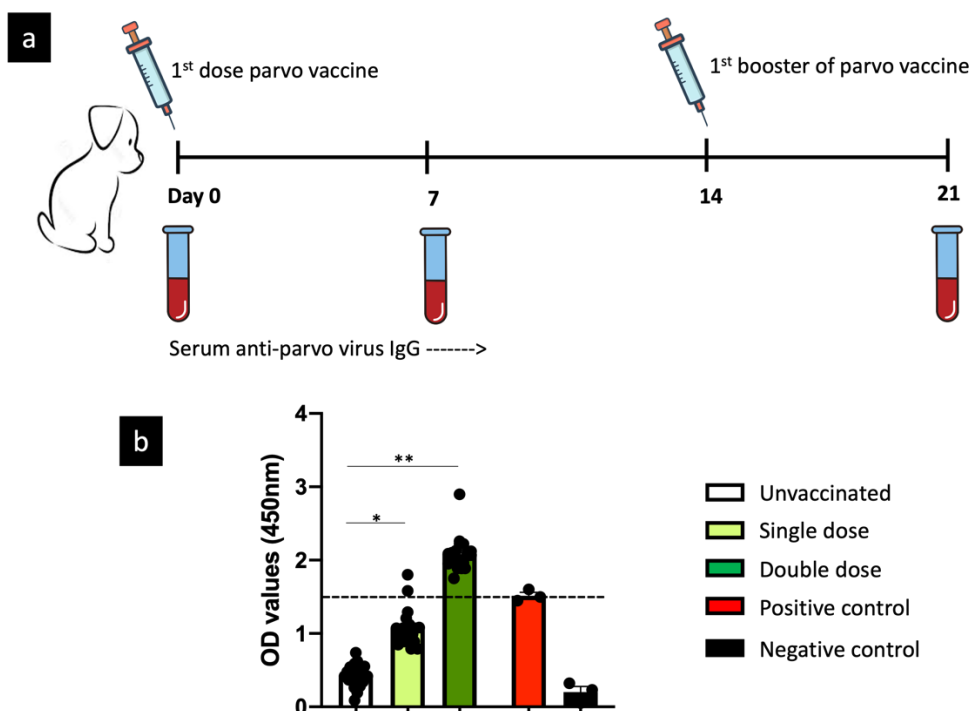


Figure 1. Experimental design and anti-parvo virus serum IgG titer. Figure 1a illustrates the study design with plan for vaccination and blood collection. Puppies of 45-180 days old were injected with Nobivac Dog® at day 0 and 14 (a). Blood samples were collected at day 0 before injection and at day 7 and 21. Optical density values of the serum from vaccinated and unvaccinated dogs was measure using ELISA (b). Sera from the unvaccinated dogs and negative control of the ELISA kits showed the lowest OD values. The sera from dogs received one booster immunization with canine parvo virus vaccine showed the highest OD values. The dashed line corresponds to the OD of the positive control so that all samples above the dashed line are positive.

Note: Statistical difference by unpaired t test, $p < 0.05$, $** < 0.01$.

3.3. Anti-Parvo Virus IgG Titer Was Diverse among the Individual Category of Dogs

We investigated the individual variation of dogs within the double-dose vaccination induced anti-parvo virus IgG. We observed that the female dogs generated significantly higher anti-IgG following two-dosage of parvo vaccination compared to male dogs Figure 2. Analysis of antibody titer also revealed that the pure-bred dogs had the highest amount of IgG followed by the cross-bred and non-descriptive local dogs. The dogs with young age

responded to the parvo-vaccine poorer than the aged dogs of older than three-months. We didn't find any significant difference in antibody generation among companion, pet, and stray dogs.

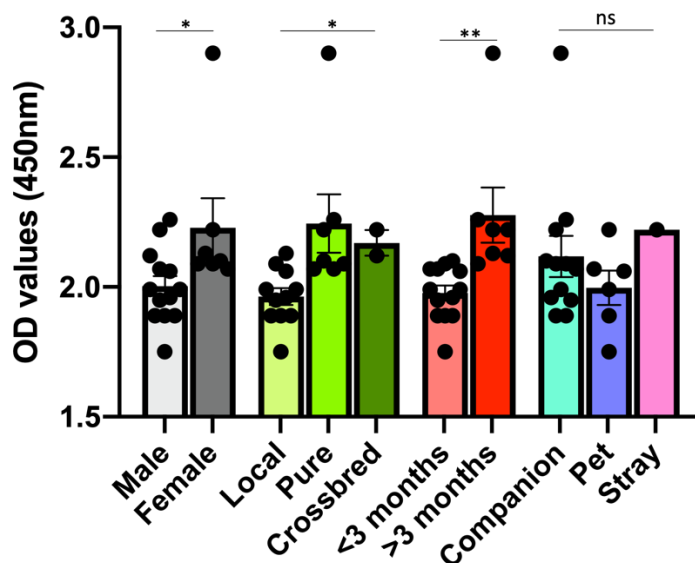


Figure 2. Serum anti-parvo virus IgG among individual category of dogs. Anti-parvo virus IgG was significantly higher in female than male (unpaired t test). The difference among local, pure bred and cross-bred dogs was also significant (ANOVA). Older dogs had higher titer than youngsters of less than three-months age (unpaired t test). The IgG titer was not significantly different among companion, pet, and stray dogs (ANOVA).
Note: $p < 0.05$, $** < 0.01$, ns: not significant.

4. DISCUSSION

We aimed to investigate the parvo-virus vaccine induced serum IgG levels in dogs following single or double dose of injections. The anti-Parvo virus antibody levels were also compared between age, sex, source and whether the participant dogs were kept as a companion or just as pet. The demographic data revealed that purebred male dogs are the primary choice by the people in this area. Male dogs are more faithful as a guard and free from risk of breeding, maybe the reason behind popularity [25]. Purebred dogs retain originality of the breed characteristics [26]. Adopted dogs usually have proper history of breeding and vaccination, previous illness, feeding, and management known making them more attractive by the interested dog owners [27]. Vaccination at early age suggested that the owners are conscious enough about the health of their pets as most dogs are kept as companions.

We performed ELISA to determine serum IgG titer against Parvo virus type 2 before starting immunizations and following single dose or double dose of vaccination. ELISA is the Gold Standard diagnostic test for the detection of antibodies in serum and other body fluids [28]. It was observed that the baseline sera had some anti-Parvo virus IgG. This could be due to maternal antibody transfer that was sustained in the puppies while we collected samples [29]. Findings of the current study also suggest that the dogs received single or double dose of parvo virus vaccine induced IgG generation, indicating the presence of IgG-inducible parvo virus antigen in the vaccine Nobivac Dog® that were maintained proper cool chain during transportation from lab to dog. However, single injection of vaccination did not induce IgG at protective level. This suggests that single dose recipients are not fully protected from natural infections and are still at risk [30]. On the contrary, the dogs received two-separate shots of injections generated enough IgG to protect the dogs Figure 1b. This is the exciting findings of the current study and indicates that to get protective level of anti-Parvo virus IgG at least two-shots are essential (Figure 3). This finding is similar to current Covid 19 vaccination where at least two shots of immunizations are recommended in many countries of the world to get enough neutralising antibodies against Corona virus [31]. However, how long the antibodies sustain in the blood at a protective level has not been assessed. Vaccination with individual Parvo virus type 2 should also been assessed instead of combined vaccination. To get the *in-situ* levels of

protective and neutralizing anti-Parvo virus IgG, hemagglutination inhibition test should be performed rendering limitation of the current investigation [30].

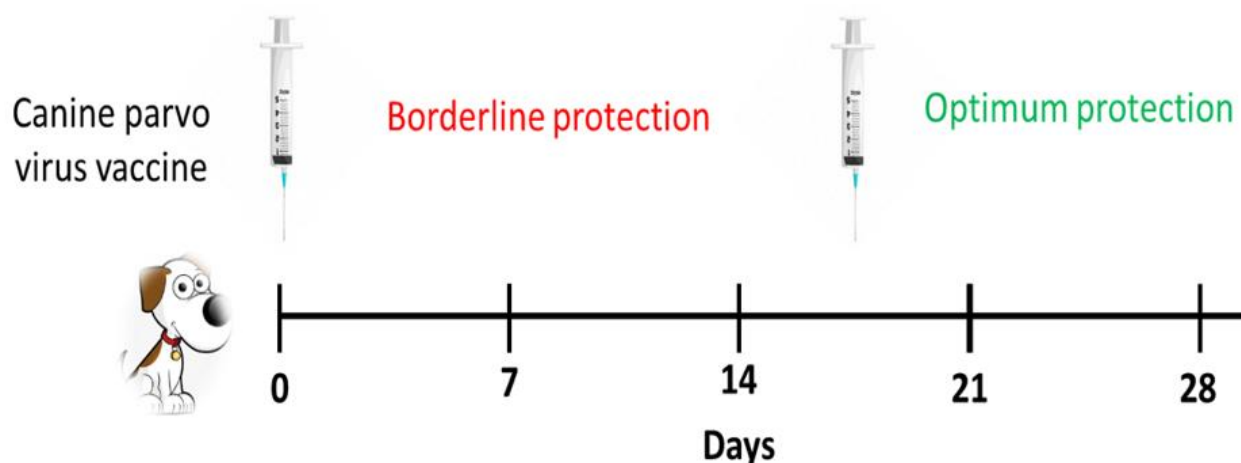


Figure 3. Redesign of effective immunization schedule for canine parvo virus infection. Primary vaccination with single dose of the vaccine provides only borderline protection. Optimum protection against canine parvo virus needs at least one booster injection 2-3 weeks following the primary vaccination.

The demographic comparison with anti-Parvo virus antibody titer indicates that female dogs generated higher level of IgG probably due to the intrinsic ability of female dogs. In a study on dogs in Japan, Taguchi, et al. [32] observed no difference between the male and females in generating serum IgG against canine Parvo virus. We also observed higher antibody in purebred followed by crossbred and lowest titers in the local stray population. The stray dogs live with poor hygiene and constantly get infections from the environment, may be the reason for low level of anti-Parvo IgG generation. Our findings also observed higher antibody titers in aged dogs and is opposite to the findings reported by Taguchi, et al. [32]. The low titer in young puppies might be due to transfer of maternal antibodies that could probably neutralized the vaccine injected.

The current study demonstrated that single injection of parvo virus vaccine in dogs does not provide optimum protection against the disease. However, a booster injection with 2-3 weeks apart from the primary vaccination provides enough serum antibody generation to fight the natural infection. In a nutshell, vaccination schedule should be followed by the veterinarians as well as by the dog owners is at least one booster injection following the primary vaccination to get effective prevention and control of canine parvo viral infection. The schedule is like the current vaccination schedule in human to fight Covid 19.

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REFERENCES

- [1] M. Hossain, K. Ahmed, A. S. P. Marma, S. Hossain, M. A. Ali, A. K. M. Shamsuzzaman, and A. Nishizono, "A survey of the dog population in rural Bangladesh," *Preventive Veterinary Medicine*, vol. 111, pp. 134-138, 2013. Available at: <https://doi.org/10.1016/j.prevetmed.2013.03.008>.
- [2] N. Runa, S. Ahmed, A. Husna, N. Runa, S. Yesmin, and A. Badruzzaman, "Prevalence of diseases in pet animals at Dhaka city of Bangladesh," *Annals of Veterinary and Animal Science*, vol. 3, pp. 88-92, 2016.

- [3] L. M. Freeman, S. K. Abood, A. J. Fascetti, L. M. Fleeman, K. E. Michel, D. P. Laflamme, C. Bauer, B. L. Kemp, J. R. Van Doren, and K. N. Willoughby, "Disease prevalence among dogs and cats in the United States and Australia and proportions of dogs and cats that receive therapeutic diets or dietary supplements," *Journal of the American Veterinary Medical Association*, vol. 229, pp. 531-534, 2006. Available at: <https://doi.org/10.2460/javma.229.4.531>.
- [4] M. Banyard, "Prevalence of intercurrent disease in dogs and cats presented for vaccination at a veterinary practice," *Australian Veterinary Journal*, vol. 76, pp. 600-601, 1998. Available at: <https://doi.org/10.1111/j.1751-0813.1998.tb10236.x>.
- [5] M. Tarafder and M. Samad, "Prevalence of clinical diseases of pet dogs and risk perception of zoonotic infection by dog owners in Bangladesh," *Bangladesh Journal of Veterinary Medicine*, vol. 8, pp. 163-174, 2010. Available at: <https://doi.org/10.3329/bjvm.v8i2.11201>.
- [6] D. H. Molyneux, "Neglected diseases but unrecognised successes—challenges and opportunities for infectious disease control," *The Lancet*, vol. 364, pp. 380-383, 2004. Available at: [https://doi.org/10.1016/s0140-6736\(04\)16728-7](https://doi.org/10.1016/s0140-6736(04)16728-7).
- [7] I. Robertson, P. Irwin, A. Lymbery, and R. Thompson, "The role of companion animals in the emergence of parasitic zoonoses," *International Journal for Parasitology*, vol. 30, pp. 1369-1377, 2000. Available at: [https://doi.org/10.1016/s0020-7519\(00\)00134-x](https://doi.org/10.1016/s0020-7519(00)00134-x).
- [8] A. Kornblatt and P. Schantz, "Veterinary and public health considerations in canine roundworm control: A survey of practicing veterinarians," *Journal of the American Veterinary Medical Association*, vol. 177, pp. 1212-1215, 1980.
- [9] B. Dodet, A. Goswami, A. Gunasekera, F. de Guzman, S. Jamali, C. Montalban, W. Purba, B. Quiambao, N. Salahuddin, and G. Sampath, "Rabies awareness in eight Asian countries," *Vaccine*, vol. 26, pp. 6344-6348, 2008. Available at: <https://doi.org/10.1016/j.vaccine.2008.09.003>.
- [10] Y. Hercules, N. J. Bryant, R. M. Wallace, R. Nelson, G. Palumbo, J. N. Williams, J. M. Ocana, S. Shapiro, H. Leavitt, and S. Slavinsk, "Rabies in a dog imported from Egypt—connecticut, 2017," *Morbidity and Mortality Weekly Report*, vol. 67, pp. 1388-1391, 2018. Available at: <https://doi.org/10.15585/mmwr.mm6750a3>.
- [11] U. U. Eze, E. Ngoepe, B. M. Anene, R. C. Ezeokonkwo, C. Nwosuh, and C. T. Sabeta, "Detection of lyssavirus antigen and antibody levels among apparently healthy and suspected rabid dogs in South-Eastern Nigeria," *BMC Research Notes*, vol. 11, pp. 1-6, 2018. Available at: <https://doi.org/10.1186/s13104-018-4024-z>.
- [12] L. Thomsett, "Diseases transmitted to man by dogs and cats," *The Practitioner*, vol. 191, pp. 630-640, 1963.
- [13] N. Nikitiuk, "Role of animals and birds as sources of Salmonella infections in man (literature review)," *Journal of Microbiology, Epidemiology and Immunobiology*, vol. 47, pp. 123-130, 1970.
- [14] B. Dada, D. Adegboye, and A. Mohammed, "A survey of gastro intestinal helminth parasites of stray dogs in Zaria, Nigeria," *The Veterinary Record*, vol. 104, pp. 145-146, 1979. Available at: <https://doi.org/10.1136/vr.104.7.145>.
- [15] CDC, "Center for disease control and prevention. Retrieved from: <https://www.cdc.gov/onehealth/basics/zoonotic-diseases.html>," 2018.
- [16] WHO, "Strategic framework for elimination of human rabies transmitted by dogs in the South-East Asia Region. Retrieved: <https://www.who.int/publications/i/item/strategic-framework-for-elimination-of-human-rabies-transmitted-by-dogs-in-the-south-east-asia-region>," 2017.
- [17] M. J. Day, M. Horzinek, R. Schultz, and R. Squires, "WSAVA Guidelines for the vaccination of dogs and cats," *The Journal of Small Animal Practice*, vol. 57, pp. 4-8, 2016. Available at: <https://doi.org/10.1111/jsap.12431>.
- [18] S. Newbury, M. K. Blinn, P. A. Bushby, C. B. Cox, J. D. Dinnage, B. Griffin, K. F. Hurley, N. Isaza, W. Jones, and L. Miller, "Guidelines for standards of care in animal shelters," *Association of Shelter Veterinarians*, pp. 1-64, 2010.
- [19] J. W. Stull, A. P. Carr, B. B. Chomel, R. D. Berghaus, and D. W. Hird, "Small animal deworming protocols, client education, and veterinarian perception of zoonotic parasites in western Canada," *The Canadian Veterinary Journal*, vol. 48, pp. 269-276, 2007.
- [20] R. Ford, L. Larson, R. Schultz, and L. Welborn, "2017 AAHA canine vaccination guidelines," *Journal of the American Animal Hospital Association*, vol. 53, pp. 243-251, 2017.

- [21] W.-J. Song, H.-T. Kim, H.-S. Yoo, and H.-Y. Youn, "Guidelines for vaccination of dogs and cats in Korea," *Clinical and Experimental Vaccine Research*, vol. 3, pp. 244-247, 2014. Available at: <https://doi.org/10.7774/cevr.2014.3.2.244>.
- [22] AVA, "Vaccination of dogs and cats by Australian veterinary association. Retrieved from: <https://www.ava.com.au/policy-advocacy/policies/companion-animals-health/vaccination-of-dogs-and-cats/>," 2018.
- [23] CVMA, "Vaccination and your cat by Canadian veterinary medical association. Retrieved from: <https://www.canadianveterinarians.net/related-resources/vaccination-and-your-cat/>," 2017.
- [24] N. Salvi, R. Deopurkar, A. Waghmare, M. Khadilkar, M. Kalolikar, S. Gade, and L. Mohite, "Validation of indirect Elisa for quantitative testing of rabies antibodies during production of antirabies serum using equines," *Procedia in Vaccinology*, vol. 2, pp. 3-11, 2010. Available at: <https://doi.org/10.1016/j.provac.2010.03.001>.
- [25] H. R. Stone, P. D. McGreevy, M. J. Starling, and B. Forkman, "Associations between domestic-dog morphology and behaviour scores in the dog mentality assessment," *PloS One*, vol. 11, p. e0149403, 2016. Available at: <https://doi.org/10.1371/journal.pone.0149403>.
- [26] H. G. Parker, L. V. Kim, N. B. Sutter, S. Carlson, T. D. Lorentzen, T. B. Malek, G. S. Johnson, H. B. DeFrance, E. A. Ostrander, and L. Kruglyak, "Genetic structure of the purebred domestic dog," *Science*, vol. 304, pp. 1160-1164, 2004. Available at: <https://doi.org/10.1126/science.1097406>.
- [27] S. Corsetti, L. Pimpolari, and E. Natoli, "How different personalities affect the reaction to adoption of dogs adopted from a shelter," *Animals*, vol. 11, p. 1816, 2021. Available at: <https://doi.org/10.3390/ani11061816>.
- [28] C. Sonmez, N. Coplu, A. Gozalan, L. Akin, and B. Esen, "Comparison of in-house biotin-avidin tetanus IgG enzyme-linked-immunosorbent assay (ELISA) with gold standard in vivo mouse neutralization test for the detection of low level antibodies," *Journal of Immunological Methods*, vol. 445, pp. 67-70, 2017. Available at: <https://doi.org/10.1016/j.jim.2017.03.012>.
- [29] J. Wilson and W. Hermann-Dekkers, "Experiments with a homologous, inactivated canine parvovirus vaccine in vaccination programmes for dogs," *Veterinary Quarterly*, vol. 4, pp. 108-116, 1982. Available at: <https://doi.org/10.1080/01652176.1982.9693850>.
- [30] A. Rota, A. Dogliero, E. Muratore, P. Pregel, A. Del Carro, and L. Masoero, "Serological survey of canine parvovirus 2 antibody titres in breeding kennels in northern Italy," *BMC Veterinary Research*, vol. 15, pp. 1-5, 2019. Available at: <https://doi.org/10.1186/s12917-019-2085-4>.
- [31] S. Chenchula, P. Karunakaran, S. Sharma, and M. Chavan, "Current evidence on efficacy of COVID-19 booster dose vaccination against the Omicron variant: A systematic review," *Journal of Medical Virology*, vol. 94, pp. 2969-2976, 2022. Available at: <https://doi.org/10.1002/jmv.27697>.
- [32] M. Taguchi, K. Namikawa, T. Maruo, K. Orito, J. Lynch, and H. Sahara, "Antibody titers for canine parvovirus type-2, canine distemper virus, and canine adenovirus type-1 in adult household dogs," *Canadian Veterinary Journal*, vol. 52, pp. 983-986, 2011.

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