Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 48, Number 1, p. 57-66, March 2020
DOI: 10.21608/zvjz.2019.15808.1072

RESEARCH ARTICLE

The Efficacy of PIND-ORF with Canine ParvoVirus vaccines in the protection of experimentally challenged puppies against the newly identified CPV-2a virus

Ali A. Abdel-Rhman[1]*, Farouk A. El Balkemy[2], Nasser Z. Abouzeid[2] and Samir M. Edries[3]
[1] Prison Sector, Ministry of Interior, Cairo, Egypt
[2]Animal Medicine Department, Infectious Diseases, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt
[3]Veterinary Serum and Vaccine Research Institute, Abassia, Cairo, Egypt

Article History: Received: 08/08/2019 Received in revised form: 04/09/2019 Accepted: 15/09 /2019

Abstract

Canine parvovirus infection (CPV) is one among several hazard diseases that incorporates a dramatic end and remains a common and vital reason of morbidity and mortality in puppies. The aim of this study is to evaluate the efficacy of local and imported CPV vaccines along with PIND-ORF against the experimentally challenged puppies with the newly identified CPV-2a virus. Forty native breed puppies around 45 days old free from internal and external parasites (as examined clinically) and negative for CPV antibodies (as screened by serum neutralization test) were enrolled, and randomized into five groups (8 animals, each). Vaccinated groups were compared to each other, or to paramunity inducer inoculated group and to neither treatment nor vaccinated group. It was found that both local canine parvovirus and Vanguard are safe and potent vaccines inducing no clinical post-vaccination reaction and high levels of specific CPV antibodies (256) by 30 days post-vaccination. Such vaccines provided 80% protection for challenging puppies against the recent virulent strain (CPV-2a), while, unvaccinated puppies did not withstand the challenging virus infection. Besides, it was noticed that PIND-ORF enhanced the puppies immune response through the 1st 2 weeks post-vaccination, however, it was unable to enhance their ability to guard against the new CPV-2a virus infection strain. So it may be concluded that the currently used CPV vaccines; either the local or imported ones can protect puppies towards new CPV-2a strain along with paramunity inducer PIND-ORF.

Keywords: Canine parvovirus, Puppies, Enteritis, PIND-ORF, Vaccine.

Introduction

Canine parvovirus (CPV) infection is a highly infectious disease acquired through the fecal-oral route. The infection is established by replication of the virus in quickly proliferating cells, as lymphoid tissues, intestinal crypt epithelial cells, precursor cells in the bone marrow and seldom myocardium in puppies through the first two weeks of life [1, 2]. Intestinal tract damage increments the hazard of bacterial translocation and consequent coliform septicemia, which may lead to the development of a systemic inflammatory response that can progress to septic shock and eventually death [3]. Lacking immunization against parvovirus during the primary year of life is an extra hazard factor for the infection [4]. Canine parovirus (CPV) belongs to genus Parvovirus and has been counted within the special species Feline panleukopenia virus along side raccoon parvovirus (RPV) and the mink enteritis virus (MEV) [5]. The control of CPV-2 is a worldwide challenge, in any case, the foremost effective strategy of control is immunization. The vaccine based on the original antigenic sort CPV-2, have been appeared to secure puppies against disease with the modern (CPV-2a/2b) antigenic types [6]. It is well known that the ideal for vaccines is to contain the most recent antigenic types of a given virus to provide the most total

*Corresponding author e-mail: (aliahmed@vet.zu.edu.eg), Sector Ministry of Interior, Cairo, Egypt.

protection, although the new vaccines are as immunogenic as the old ones [7]. Attenuated CPV vaccines given great protection and long resistance [8]. Right now, the attenuated vaccines are determined from either CPV-2b isolates or the original type-2 virus. There is an alarm that the puppies vaccinations used right now to avoid CPV disease may not provide successful security against the new variants of CPV type 2 [9]. In spite of the truth that antigenic types CPV-2a, 2b and 2c were replaced totally the first CPV-2 type, most commercial vaccines are still utilized the first CPV-2 type. Different studies have illustrated that protection against CPV-2 variants are still viable by using CPV-2 vaccines [8,10]. There have been a number of reports focusing on the need to upgrade the CPV-2 vaccines by supplanting the original CPV type 2 (which has experienced extinction) with the CPV-2 variations currently circulating in nearby canine populations. The prophylaxis against CPV could be progress by using Polyvalent CPV vaccines [7,11,12]. A differences of harmful impacts, such as infectious pathogens, malignant cells, poisons, and foreign substances could be limited by paramunity which use as non-pathogen-specific and non-antigen-specific security of term. The innate immune system is reportable to stimulateby the paramunity inducer PIND-ORF and, in the event that utilized as a supplementary pharmaceutical, may cause a more fast advancement in clinical indications in puppies with CPV infection [13]. Puppies infected by CPV that lack specific immunity might be beneficial to use paramunity inducers so decreasing the hazard of extreme sickness and quickening recovery. Paramunity inducers are non-immunizing biological products with a paraspecific impact on the innate immune system, stimulating monocytes and macrophages that upgrade the rate of phagocytosis, progressing the action of other lymphoreticular cells and common killer cells boosting the work of spontaneous cell-mediated cytotoxicity. Paramunity inducers moreover can improve release, production, and interaction of numerous cytokines, such as tumor necrosis factor (TNF)-α, IFN-α, IFN-γ, interleukin (IL)-2, and granulocyte-macrophage colony-stimulating factor (GM-CSF) [14,15]. PIND-ORF is authorized for utilize in Germany, the Czech Republic, and Slovakia in puppies and in other animal species, it has been detailed to be viable when utilized therapeutically or metaphylactically [16, 17]. In any case, a few other organized studies assessing the therapeutic viability of PIND-ORF in feline leukemia virus disease or in post-weaning multisystemic squandering disorder in pigs may not illustrate a advantageous impact [18, 19]. The present work aims to evaluate the efficacy of local and imported CP vaccines and to any extent they can withstand the infection with the newly identified CPV-2a virus, in addition to investigate the efficacy of PIND-ORF (zylexis) in vaccinated and experimentally infected puppies.

Materials and Methods

This study has been conducted in accordance with the guidelines of the Zagazig University IACUC Committee, approval number ZU-IACUC/3/S/29/2018.

Grouping of animals for experimental study

Forty native breed puppies aged around 45 days were clinically examined. The inclusion criteria included puppies free from internal and external parasites and negative for CP antibodies (by serum neutralization test). Those animals were randomly allocated into 5 groups (8 animals /group), animals in group-1 were vaccinated with the local CP vaccine using a dose of $10^3 TCID_{50}$/animal (1ml) via subcutaneous (SC) route. While group-2 were vaccinated with Vanguard vaccine, each experimentally vaccinated puppy received $3\log_{10}$ $TCID_{50}$ (1ml) of the live attenuated CPV vaccine inoculated SC according to Kotb [20]. Group-3 were vaccinated with the local CP vaccine along with PIND-ORF using a dose of 1ml SC on 0, 2 and 4 days post-vaccination with the local CP vaccine. The animals in group-4 were inoculated with PIND-ORF only, meanwhile, puppies in group-5 were kept without vaccination or treatment under strict hygienic measures as a control group.

Sampling

Blood samples

Blood samples (4mL) were collected from cephalic vein of the fore limb of puppies (n= 40) under the study experiment and then dispersed in plain tubes for separation of serum and stored at -20C according to Feldman *et al.* [21] until used for serum neutralization test. A cold container used to keep those samples during transportation to the Department of Pet Animal Vaccine Research (DPAVR); Veterinary Serum and Vaccine Research Institute (VSVRI) Abassia, Cairo, Egypt. From all animal groups, serum samples were obtained weekly up to 4 weeks then every month up to 6 months of vaccination for following up the levels of induced antibodies.

Faecal samples

Faecal samples were collected from all puppies for virus isolation under the study design and then examined for internal parasites according to Hendrix and Robinson [22].

Vaccines

Live attenuated locally produced CP vaccine was provided by the Department of Pet Animal Vaccine Research (DPAVR); Veterinary Serum and Vaccine Research Institute (VSVRI) Abassia, Cairo. Vanguard vaccine (VANGUARD Plus 5) was supplied by ZOETIS EGYPT, New Cairo, Egypt and used for vaccination of experimental puppies. Vanguard vaccine is applied for vaccination of 6 weeks old and older puppies against canine distemper (CD), canine infectious hepatitis (CVA-1), respiratory diseases caused by canine adenovirus 2(CVA-2), canine parainfluenza (CPIV) and canine parvovirus (CPV-2c).

Canine parvovirus (CPV)

The newly isolated and identified CPV-2a with accession number MK614454 was used to challenge experimentally vaccinated puppies with the local vaccine.

PIND-ORF (Zylexis)

PIND-ORF (Zylexis) containing inactivated P. ovis strain D 1701 (with a minimum of 230 IFN units) was supplied by Zoetis and used on days 0, 2, and 4 day. Dose, frequency, and route of admission were chosen according to the manufacturer's recommendations. The used dose was 1ml/puppy inoculated subcutaneously.

Antisera

Canine Parvovirus antiserum

Specific anti-canine parvovirus serum was kindly supplied by the Department of Pet Animal Vaccine Research, Serum and Vaccine Research Institute, Abbasia, Cairo and used for virus identification through application of virus neutralization test.

Canine Parvovirus antiserum conjugated with fluorescin isothiocyanate

It was kindly supplied by the Department of Pet Animal Vaccine Research, Serum and Vaccine Research Institute, Abbasia, Cairo. It used for identification of the virus isolates using direct fluorescent antibody technique.

Challenge test

Challenge of expermental animals after 21 days of vaccination (5 of each group) were carried out through the intranasal route using $10^5 TCID_{50}$ (1ml) of the virulent recent CPV-2a strain in step with Kotb [20]. The other three puppies in each group were kept for following up the levels of exhibited CPV antibodies and as test control.

Serum Neutralization test (SNT)

SNT was carried out on serum samples obtained from all experimental puppies before and at weekly intervals up to 4 weeks then every month up to 6 months post application of the experimental work using the micro titer technique according to Bass *et al.* [23]. The titer of antibody was expressed as the complementary of the ultimate serum dilution which neutralized and inhibited completely the CPE of 100 $TCID_{50}$ of the used virus according to Singh *et al.* [24].

Statistical analysis

Microsoft Excel program was used to analyze all information collected through history, basic clinical examination, laboratory examinations and result measures recorded. Information were at that point imported into Statistical Package for the Social Sciences (SPSS form 20.0) software for analysis. Concurring to the sort of data subjective represent as number and percentage, quantitative proceeds group represent by mean ± SD, the following tests were utilized to test differences for significance, difference and association of qualitative variable by Chi square test (X2) or Fisher exact. ANOVA for multiple quantitative continues data. P value was set at <0.05 for significant results and <0.001 for high significant result.

Results and Discussion

The obtained results revealed that all vaccinated puppies did not show any abnormal clinical symptoms post vaccination that indicate the safety of used vaccines and PIND-ORF. Such animals exhibited detectable specific CP antibodies by the first week specially in local vaccinated group but it was noticed that puppies received PIND-ORF with the local vaccine showed higher antibody titers 8 than those received either the local or Vanguard vaccine alone (4 and 0 respectively). As depicted in Table 1, antibody titers of CP increased earlier in animals inoculated with PIND-ORF than in other vaccinated ones although the peak antibody titer was the same in all groups (256) by the 3rd to the 4th month post vaccination and retained at this level up to 6 months post vaccination

Table (1): Canine parvo serum neutralizing antibody titers in vaccinated puppies

Post vaccination intervals	Mean CP serum neutralizing antibody titer* in vaccinated puppies				
	The native vaccine	Vanguard vaccine	native vaccine with PIND-ORF	Unvaccinated control #	P
1st W pv**	4 (2-6)	0 (0-0)	8 (4-16)		0.001**
2nd Wpv	8 (4-16)	4 (2-8)	16 (4-32)		0.00**
3rd W pv	16 (8-32)	16 (8-32)	32 (16-64)		0.0002**
4th Wpv	64 (32-128)	32 (16-64)	128 (64-256)		0.00**
2nd Mpv***	128 (64-256)	64 (32-128)	256 (128-256)		0.00**
3rd Mpv	256 (256-256)	128 (64-256)	256 (256-256)	↑ 0 ↓	0.021*
4th Mpv	256 (256-256)	256 (256-256)	256 (256-256)		---------
5th Mpv	256 (256-256)	256 (256-256)	256 (256-256)		-------
6th Mpv	256 (256-256)	256 (256-256)	256 (256-256)		--------
	0.00**	0.00**	0.00**		

* CP serum neutralizing antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited completely the CPE of 100 $TCID_{50}$ of CPV

**Wpv= week post vaccination ***Mpv= month post vaccination

unvaccinated control= group treated with PIND-ORF and unvaccinated, plus group untreated and unvaccinated.

These findings could be explained in a parallel manner to those reported by Spibey *et al.* [8] who stated that attenuated CPV vaccines provided excellent protection and a longer immunity. Regarding the used CPV strains included in the applied vaccination; in spite of the fact that the antigenic types CPV-2a, 2b and 2c totally replaced the original CPV-2 type which is utilized in many commercial vaccines, it was concluded that CPV-2 vaccines are still successful stimulating protection against CPV-2 variants in puppies [8, 10]. Monovalent CPV-2 vaccines are broadly prescribed for initial immunization of puppies and moreover accessible containing exceptionally high titer virus (107 TCID50) [7]. Around 60% of all puppies seroconverted after a single vaccination either at 8 weeks of age with a multivalent vaccine or at 6 weeks of age with a CPV monovalent vaccine. Right now, accessible vaccines based on CPV-2 and CPV-2b safeguard against all known strains of CPV, including the current CPV-2c strain [10]. Since the recognizable proof of canine

parvovirus type 2, three variants have hence been observed contrasting from the historical CPV-2 and each other just by 1–2 amino acids [25]. They explored whether cross-reaction was initiated by vaccination with a CPV-2b containing vaccine.

Antibody profile and serum analysis from challenge puppies with CPV-2b vaccinated with a multivalent vaccine gave similar antibody profiles for CPV2 and serological responses were higher than those for CPV2. In any case, CPV-2 responses were considered protective clinically. After vaccination puppies showed up a speedy increase in antibody titers, after second vaccination come to a plateau with a slight diminish to challenge after which speedy anamnestic reactions were seen. Serological responses evaluation proposes inoculation with CPV-2b would cross-protect extinct within the field. They concluded that vaccination against all currently circulating field strains, CPV-2a and CPV-2c, and the extinct field strain CPV-2 with a multivalent vaccine containing the CPV-2b variant strain will initiate cross-reactive serological reactions in puppies. With respect to Egypt, a live attenuated CP vaccine was arranged effectively actuating high long enduring immunity in vaccinated puppies [20]. A bivalent live attenuated cell culture vaccine was arranged against canine distemper and canine parvo viruses. This vaccine was found to be secure and powerful as illustrated by serum neutralization and challenge tests [26]. These vaccines create varying levels of defensive immunity and are secure either alone or in combination with other vaccine components [27, 28]. It was reported that PIND-ORF stimulates the innate immune system that explains the earlier and higher starting of induced CP antibodies in puppies received it with the local vaccine than in puppies received the vaccines only. This is in context with Mayr and Mayr [14] who stated that the innate immune paraspecific impacted by paramunity inducers which are non-immunizing biological items. They also concluded that paramunity inducers stimulating monocytes and macrophages lead to upgrade the rate of phagocytosis and progressing the activity of other lymphoreticular cells.

Table (2): Protection efficacy of Canine Parvo vaccines and PIND-ORF against experimental Canine Parvovirus Infection.

Puppies groups	Number of test puppies	Number of sick puppies	Number of dead puppies	Protection %
Vaccinated with the local CP vaccine	5	1 (20.0%)	0 (0.0%)	80
Vaccinated with Vanguard vaccine	5	1 (20.0%)	0 (0.0%)	80
local CP vaccine with PIND-ORF	5	1 (20.0%)	0 (0.0%)	80
Treated with PIND-ORF	5	4 (80.0%)*	1 (20.0%)*	20
Unvaccinated untreated	5	5 (100.0%)*	4 (80.0%) #	0
P		0.00**	0.00**	

* P≤0. 05 ** P≤0. 01

As demonstrated in Table 2, the challenge of vaccinated puppies against the isolated CPV strain (CPV a2) showed a protection rate of 80%, each in puppies vaccinated with the local, Vanguard, local with PIND-ORF, respectively and protection rates of 20% and 0% in PIND-ORF treated and unvaccinated control puppies, respectively. These results appear to be supported by those of Yule *et al.* [6] who concluded that the infection with the new (CPV-2a/2b) can be avoid by vaccine based on the original antigenic type CPV-2, and Spibey *et al.* [8] who stated that attenuated CPV vaccines provided excellent protection and a longer immunity. In addition, Spibey *et al.* [8] and Larson and Schultz [10] reported that the original CPV-2 vaccines are still viable in stimulating protection against CPV-2 variants although the antigenic variants CPV-2a, 2b and 2c was totally replaced the CPV-2

type. On the other side, it was found that PIND-ORF induced higher survival rate in treating puppies (20%) than in untreated unvaccinated puppies (0%). This is in agreement with Proksch *et al.* [13] who reported that the harmful impacts, such as infectious pathogens, malignant cells, poisons, and foreign substances could be limited by using paramunity which work as non-pathogen-specific and non-antigen-specific security of term. The innate immune system is reportable to stimulate by the paramunity inducer PIND-ORF and, in the event that utilized as a supplementary pharmaceutical, may cause a more fast advancement in clinical indications in puppies with CPV infection [16, 17].

Figure (1): Puppy suffering from CPV infection showing weakness, dullness and vomiting.

Figure (2): A weak puppy suffering from bloody diarrhea.

Figure (3): Severe congestion of the internal organs of dead puppy infected with CPV five days post infection in unvaccinated untreated group.

Affected puppies with the challenge virus showed fever, vomiting, bloody diarrhea, dehydration and rough hairs and some of these puppies were dead showing sever congestion of the internal organs and enlarged spleen as shown in Figures (1, 2, 3). This was in concordance with the results of previous studies [29, 30] who stated that bloody diarrhea was one of the most clearly observed signs in 40% of CPV infected puppies. It was concluded that vomiting is one of the major clinical sign in infected puppies developed early within 24 to 48 post infection. This was in concordance with the results of previous studies [2, 31, 32]. On the other hand, post mortem findings came similar to and confirmed by those previously reported [33, 34]. In addition sequence analysis of recent isolates showed that they are closely related to CPV-2a genotype. CPV-2a and 2b genotypes that were detected in Egypt [35- 38]. Also, El-Gendy [37] concluded that recent field CPV strains are 99% homologous with the local vaccine strain in Egypt and there is no need to change the current used vaccine strains.

Conclusion

The recurrently used CPV vaccines; either the native or imported ones; can guard puppies against new CPV-2a strain. PIND-ORF could be used to accelerate and enhance the induction of CP antibodies in vaccinated puppies and may reduce the hazard of virus infection in affected animals.

Acknowledgments

This work was done without funding from any organization. Necessary facilities of the Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Department of Pet Animal Vaccine Research (DPAVR); Veterinary Serum and Vaccine Research Institute (VSVRI) Abassia, Cairo,Egypt were used.

Conflict of interest

The authors have no conflict of interest to declare.

References

[1] Goddard, A. and Leisewitz, A.L. (2010): Canine parvovirus. Vet Clin North Am Small Anim Pract, 40: 1041-1053.

[2] Prittie, J. (2004): Canine parvoviral enteritis: a review of diagnosis, management and prevention. J. Vet Emerg Crit Care, 13: 167–176.

[3] Schoeman, J.P.; Goddard, A. and Leisewitz, A.L. (2013): Biomarkers in canine parvovirus enteritis, New Zealand. Vet J, 61(4): 217-222.

[4] Smith-Carr, S.; Macintire, D.K. and Swango L.J. (1997): Canine parvovirosis. Part I. pathogenesis and vaccination compendium on continuing education for the practicing veterinarian, 19 (2): 125-133.

[5] Tattersall, P.; Bergoin, M.; Bloom, M.E.; Brown, K.E.; Linden, R.M.; Muzycka, N.; Parrish, C.R. and Tijssen, P. (2005): Family *Parvoviridae* in Fauquet, C.M.; Mayo, M.A.; Maniloff, J.; Desselberger, U. Ball, L.A. (Eds); Virus Taxonomy: VIIIth report of Int. Comm. Tax. V. Elsevier Academic Press.

[6] Yule, T.D.; Roth, M.B.; Dreier, K.; Johnson, A.F.; Palmer-Densmor, M. and Simmons, K. (1997): Canine parvovirus vaccine elicits protection from the inflammatory and clinical consequences of the disease. Vaccine, 15 (6-7): 720-729.

[7] Truyen, U. (2006): Evaluation of canine parvovirus- a need for new vaccines; Vet Microbiol, 117 (1): 9-13.

[8] Spibey, N.; Greenwood, N.M.; Sutton, D.; Chalmers, W.S. and Tarpey, I. (2008): Canine parvovirus type 2 vaccine protects against virulent challenge with type 2c virus; Vet Microbiol, 128 (1-2): 48-55.

[9] Altman, K.D.; Kelman, M. and Ward, M.P. (2017): Are vaccine strain, type or administration protocol risk factors for canine parvovirus vaccine failure? Vet Microbiol, 210: 8-16.

[10] Larson, L.J. and Schultz, R.D. (2008): Do two current canine parvovirus type 2 and 2b vaccine provide protection against the

new type 2c variant?. Vet Therap, 9 (2): 94-101.

[11] Martella, V.; Cavalli, A.; Decaro, N.; Elia, G.; Campolo, M.; Bozzo, G. Tarsitano, E. and Buonavoglia, C. (2005): Immunogenicity of an intranasally administrated modified live canine parvovirus type 2b in pups with maternally derived antibodies. Clin Daig Lab. Immunol, 12 (10): 1243-1245.

[12] Cavalli, A.; Martella, V.; Desario, C.; Camero, M.; Bellacicco, A. I.; De Palo, P.; Decaro, N.; Elia, G. and Buonavoglia, C. (2008): Evaluation of the antigenic relationship among canine parvovirus type 2 variants. Clin Vac Immun, 15 (3): 534- 539.

[13] Proksch, A.L.; Unterer, S.; Speck, S.; Truyen, U. and Hartmann, K. (2015): Influence of clinical and laboratory variables on faecal antigen ELISA results in puppies with canine parvovirus infection. The Vet J, 204 (3): 304-308.

[14] Mayr, A. and Mayr, B. (1999): A new concept in prophylaxis and therapy: Paramunisation by *poxvirus* inducers. Pesquisa Veterinaria Brasileira, 19 (3-4): 91–98.

[15] Fachinger, V., Schlapp, T., Strube, W., Schmeer, N., Saalmuller, A. (2000): Poxvirus induced immunostimulating effects on porcine leukocytes. Journal of Virology ,74 (17): 7943–7951.

[16] Castrucci, G.; Osburn, B.I.; Frigeri, F.; Ferrari, M.; Salvatori, D.; Lo Dico, M. and Barreca, F. (2000): The use of immunomodulators in the control of infectious bovine rhinotracheitis. Comparative Immunology, Microbiology and Infectious Diseases , 23 (3): 163–173.

[17] Ryan, C.; Giguere, S.; Fultz, L.; Long, M.T. and Crawford, P.C. (2010): Effects of two commercially available immunostimulants on leukocyte function of foals following ex vivo exposure to *Rhodococcus equi*. Vet Immunol Immunopathol, 138(3): 198–205.

[18] Block, A.; Hartmann, K.; Lutz, H. and Kraft, W. (1997): Placebo-controlled double-blind study on the efficacy of a paramunity inducer in the treatment of naturally; FeLV- infected cats. Tierarztliche Praxis, 25 (3): 261–266.

[19] Kyriakis, S.C.; Saoulidis, K.; Lekkas, S.; Miliotis, C.C.; Papoutsis, P.A. and Kennedy, S. (2002): The effects of immuno-modulation on the clinical and pathological expression of post weaning multisystemic wasting syndrome. Journal of Comp. Pathology, 126 (1):38–46

[20] Kotb, A.M. (1994): Studies on preparation of canine parvovirus vaccine. Ph.D. Thesis. Department of Virology, Faculty of Veterinary Medicine, Cairo University.

[21] Feldman, B.F.; Zinkl, J.G.; Jain N.C. and Gasper, P.E. (2000): Schalm's Veterinary Haematology, 5th Ed., Wiley Blackwell Publishing, Ames, Iowa, U.S.A., PP. 504-507.

[22] Hendrix, C.M; Robinson, E. (2012): Diagnostic parasitology for veterinary technicians ed 4, Mosby, *E-Book*. Elsevier Health Sciences

[23] Bass, E.P.; Gill, M.A. and Beckenhaur, W.H. (1982): Development of a modified live canine origin parvo virus vaccine. JAVMA, 181 (9): 909-913.

[24] Singh, K.V.; Osman, O.A.; el Cicy, I.F. and Baz, T.I. (1967): Colostral transfer of rinderpest neutralizing antibodies to offspring of vaccinated dams. Can J Comp Med Vet Sci, 31 (11): 295-298.

[25] Wilson, S.; Joanna, I.; Elisabeth S.; Catrina S.; Anne T.; Edita P.; Gordon S. and Jeremy S. (2014): Vaccination of Puppies with Canine Parvovirus Type 2b (CPV-2b) Induces Neutralising Antibody Responses to CPV-2a and CPV-2c. Vaccine 32(42):5420–24.

[26] Khodeir, M.H.; Kotb, A. M.; Guirguis, W.I. and Habashi, Y.Z. (1998): Preparation of a bivalent vaccine against canine distemper and canine parvo viruses 4th Vet. Med. Zag. Cong. : 152-160.

[27] Edries, S.M.; Kotb, A.M.; Habashi, Y.Z. and El-Nakashly, S. (1999): Some studies on preparation of a combined vaccine against rabies and canine parvo viral diseases Alex. J. Vet. Sci. 15 (3): 591-599.

[28] Salem, Z.T.; Ali, N.I.; Guirguis, W.I. and Khodeir, M.H. (2003): Modification of the inactivated trivalent vaccine (Rabies; Canine distemper and canine parvo) used for puppies to improve its immunogenicity J. Egypt Vet. Med. Ass. 63 (2): 137-146

[29] Saho, N.; Parida, G.S. and Sardar, K.K. (2007): Clinical signs based prognosis of canine parvovirus infection. Indian Vet J, 84 (6): 637-638.

[30] Kumari, V. (2011): Clinical and therapeutic studies of diarrhea in dog's . M.V.Sc. Thesis. ANGRAU, Rajendranagar, Hyderabad.

[31] Willard, M.D. (2005): Disorders of the intestinal tract. In: Nelson Rw, Couto CG, EDS. Small animal internal medicine. 3RD ED. ST Louis: Mosby; 433-435.

[32] Decaro, N.; Elia, G.; Campolo, M.; Desario, C.; Lucent, M.S.; Bellacicco, A.L. and Buonavoglia, C. (2005c): New approaches for the molecular characterization of canine parvovirus type 2 strains. J Vet Med Series B, 52: 316–319.

[33] Cooper, B.J.; Carmichael, L.E.; Apple, M.J.G. and Greisen, H. (1979): Canine viral enteritis, II. Morphological lesions in naturally occurring infection. Cornell Vet, 69 (3): 134-144.

[34] Woods, C.B.; Pollock, R.V.H. and Carmichael, L.E. (1980): Canine parvovirus enteritis. J Am Anim Hosp Assoc, 16 (12): 171-179.

[35] Morcos, I.Y. (2013): Isolation and characterization of canine parvovirus-2 in Egypt. Ph.D. Thesis.,Department of Virology, Faculty of Veterinary Medicine, Cairo University.

[36] Fouad, A.A. (2014): Molecular characterization of canine parvovirus-2 in Egypt. M.V. Sc. Thesis. Department of Virology, Faculty of Veterinary Medicine, Cairo University.

[37] El-Gendy, I.M. (2018): Isolation and molecular characterization of canine parvovirus from Cairo and Giza in the period of 2014-2015, M.V.Sc. Thesis. Department of Virology, Faculty of Veterinary Medicine, Cairo University.

[38] Abdullah, R.S. (2018): Advanced diagnosis techniques of parvovirus infection in pet animals; M. Vet. Sci. Thesis. Faculty of Veterinary Medicine, Damanhur University.

الملخص العربي

فعالية PIND-ORF مع لقاحات فيروس البارفو في حماية الجراء التي تواجه عدوى معملية باستخدام سلالة CPV-2a المعزوله حديثا

علي احمد عبد الرحمن [١]*، فاروق عطية البلكيمي[٢]، ناصر زيدان أبوزيد[٢]، سمير محمد إدريس[٣]

[١] طبيب بيطري فى قطاع السجون بوزارة الداخلية – القاهرة - مصر

[٢] قسم طب الحيوان - الأمراض المعدية - كلية الطب البيطري - جامعة الزقازيق - مصر

[٣] معهد بحوث مصل الدم واللقاحات - العباسية - القاهرة- مصر

تعد عدوى الكلاب بفيروس البارفو من بين العديد من الأمراض الخطيرة التي تتضمن نهاية درامية وتظل سببًا شائعًا وهامًا للاصابات والوفيات في الكلاب الصغيرة. ومن ثم تهدف هذه الدراسة إلى تقييم فعالية لقاحات CPV المحلية والمستوردة إلى جانب محفز المناعة (PIND-ORF) فى الكلاب التي تم اصابتها تجريبياً باستخدام السلالة الفيروسية الجديدة CPV-2a. تمت الدراسة على ٤٠ من الجراء من السلالة المحلية بعمر ٤٥ يوما تقريبا خالية من الطفيليات الداخلية والخارجية بالفحص الاكلينيكى وخالية من الاجسام المناعية باختبار قياس الاجسام المناعية، تم تقسيمها عشوائيا الى خمس مجموعات (٨ حيوانات لكل منهما).تم مقارنة المجموعات التي تم حقنها بالتحصين مقارنة مع بعضها البعض، أو مع المجموعة التى تم اعطائها المحفزالمناعة فقط والمجموعة التى لم يتم حقنها باللقاح أو العلاج. لقد وجد أن كلا من تحصينات Parvovirus المحلية و Vanguard هما لقاحات آمنة وفعالة لا تسبب أي رد فعل اكلينيكى بعد التطعيم وتعطى مستويات عالية من الأجسام المضادة لفيروس البارفو (256) بنسبة ٣٠ يومًا بعد التطعيم. هذه اللقاحات أعطت حماية بنسبة ٨٠٪ للكلاب ضد السلالة الحادة الأخيرة (CPV-2a) في حين أن الجراء غير المحصنة لم تصمد أمام العدوى الفيروسية الصعبة. إضافة إلى ذلك، لوحظ أن PIND-ORF قد عزز استجابة الكلاب المناعية خلال الأسبوعين الأولين بعد التحصين، ومع ذلك، لم يتمكن من تعزيز قدرته على الحماية من سلالة العدوى بفيروس CPV-2a الجديدة. الخلاصة فى هذه الدراسة أن لقاحات CPV المستخدمة حاليا سواء المحلية أو المستوردة يمكن أن تحمى الجراء ضد سلالة CPV-2a الجديدة جنبا إلى جنب مع محفز المناعة PIND-ORF.