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Preparation and evaluation of combined live vaccine against canine adeno-1, canine distemper and canine parvo viruses

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List of abbreviations

AAHA	The American Animal Hospital Association
BHK	Baby Hamster kidney
CAdV	Canine Adeno virus
CDV	Canine distemper virus
CPCR	Conventional polymerase chain reaction
CPE	Cytopathic effect
CPVI	Canine parvovirus infection
DPAVR	Department of Pet Animal Vaccine Research
EM	Electron microscopy
ELISA	Enzyme linked immunosorbent assay
FAO	Food and Agriculture Organization
HBSS	Hank's balanced salt solution
HRP	Horse Radish Peroxidase
ICT	Immunochromatographic test
ICH	Infectious canine hepatitis
IFA	Immunofluorescence assay
HA	Hemagglutination
HI	Hemagglutination inhibition
MAbs	Monoclonal antibodies
MDA	Maternally derived antibodies
MDCK	Madin Darby canine kidney

MEM	Minimum essential media
MLV	Modified live vaccine
MPV	Month post vaccination
RT-PCR	Reverse transcription polymerase chain reaction
SNT	Serum neutralization test
$TCID_{50}$	Tissue culture Infective Dose Fifty
Vero	African green monkey Kidney Cells
VI	Virus isolation
VSVRI	Veterinary Serum and Vaccine Research Institute
WBM	Weybridge medium
WPV	Week post vaccination

7-SUMMARY

Dogs play an important role in the general and social relations and their protection against infectious diseases is an essential aim of veterinarians through providing of specific potent vaccines.

The present thesis deals with preparation and evaluation of live attenuated single and trivalent cell culture Canine distemper; Canine infectious hepatitis and Canine parvo vaccines.

The obtained experimental results through this work revealed that:

1-All of the prepared single and trivalent CD; CA-1 and CP vaccines were free from aerobic and anaerobic bacteria; fungi and mycoplasma and did not induce any abnormal local or systemic post vaccinal reactions neither in mice nor in puppies indicating their safety.

2- Potency testing of the prepared vaccines through application of SNT and ELISA to estimate CD antibodies in vaccinated puppies; showed that vaccinated puppies with single CD (group-1) and with the trivalent CD; CA-1 and CP vaccine (group-4) exhibited detectable specific CD antibodies by the first week (2&2≤ by SNT and 0.70&0.71 $\log_{10}$ by ELIZA respectively) recording their peaks (64 by SNT &2.5 $\log_{10}$ by ELIZA in both groups) by the 2nd month and remained stable up to 12 months post vaccination while the control group (group-5) still seronegative to CD antibodies.

3-Following up the levels of CA-1 (infectious canine hepatitis) antibody levels in different vaccinated puppy's groups;serum neutralization test and indirect linked enzyme immune sorbent assay revealed that these puppies responded well to these vaccines having detectable specific CA-1 antibodies by the first week (2&4≤ by SNT and 0.50& 0.51$\log_{10}$ by

ELIZA in group-2 and 4 respectively) recording their peaks (128 by SNT and 2.50 log_{10} by ELIZA in both groups) by the 2nd month and remained unchanged up to 12 months post vaccination.

4-Detection of Canine parvo antibody levels in vaccinated puppies through application of serological tests showed that single CP vaccine induced specific CP antibodies in vaccinated puppies within the first week as 4 and 2 by SNT and 0.90 and 0.88 log_{10} by ELIZA in group-3 vaccinated with the single vaccine and group-4 vaccinated with the trivalent vaccine respectively. Peak titers of such antibodies (128 by SNT and 2.50 log_{10} by ELIZA) were recorded by the 2nd month post vaccination in both groups with negative results among the unvaccinated group (group-5).

5-There is no any antagonizing effect of any of the vaccine viral content on the immune response of vaccinated puppies to the other virus vaccine where there are no apparently significant differences between the levels of measured antibody either by SNT or by ELIZA.

6- **Regarding evaluation of young puppies 'vaccination,** following up the levels of CA-1(infectious canine hepatitis) antibody levels in vaccinated puppy's group; serum neutralization test revealed that these puppies having detectable specific CA-1 antibodies by the first week (1.6) in group-1comparedto 0 in control group recording their peaks (6.4) by the second week after the booster dose. In these puppies; detection of Canine parvo antibody levels showed specific CP antibodies in vaccinated puppies within the first week as 0.8 in group-1 compared to zero in control group. Peak titers of such antibodies (10.4) were recorded by the 2nd week after booster dose. Canine distemper antibodies in trivalent vaccinated puppies were detectable within the first week as 1.2

in group-1 compared to zero in control group. Peak titers of such antibodies (10.4) were recorded by the 2nd week after booster dose.

7-Following up the parameters of CBC and phagocytic activity and index in vaccinated young puppies did not show significant differences compared with the unvaccinated puppies' group.

8-Estimation of serum proteins show slight elevation in the globulin levels in vaccinated young puppies than in unvaccinated puppies.