INTRODUCTION

Canine parvovirus (CPV) is responsible for acute and sometimes fatal enteritis in dogs. The virus, which first appeared in late 1978 probably arose from a very closely related virus in cats, feline panleukopenia virus through a small number of mutations in the single capsid protein (Tattersall et al., 2005). CPV is a non-enveloped virus of the autonomously replicate Paroviridae family. This small icosaheiral virus has a single-stranded DNA genome (5.2 kb) with two major open reading frames, one encoding the nonstructural proteins NS1 and NS2, and the other encoding the capsid proteins VP1 and VP2 (Nakamura et al., 2004).

Modified live virus (MLV) vaccines are widely used. These vaccines are highly effective and prepared by using either the original type CPV-2 or its variant CPV-2b (Decaro et al., 2014). These may protect dogs against parvovirus infection, and almost completely safe, as post vaccinal reactions are very rarely observed. There is study showed that most dogs developing parvovirus-like diarrhea after vaccination were infected by the field virus alone, thus ruling out any reversion to virulence of the vaccine viruses (Greenwood et al., 1995).

The primary causes of failure of CPV vaccination are interfering levels of maternally derived antibodies (MDA) that are transmitted by bitches to their offspring through colostrum and, at a lesser extent, milk. Thus, in order to avoid the interference with MDA active immunization, vaccines should be administered to pups only after weaning (Lida et al., 1990).

Vaccination stimulates both humoral response via antibody production and cellular responses via B and T lymphocytes.
MATERIAL AND METHODS

EXPERIMENTAL MATERIAL
Ten batches of live attenuated canine Parvovirus vaccines were delivered to Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Abbasia-Cairo. These batches had been evaluated in the last year for sterility, safety and potency. Madin-Darby Canine Kidney (MDCK) cells were supplied by Strain Bank at CLEVB. The cells were used for IFT, SNT, vaccine titration and identification. Canine parvovirus tissue culture was adapted on MDCK with titer of 6 log_{10} TCID_{50}/ml. This virus (positive control) was used in IF technique, sensitivity test for SNAP Parvo test and SNT for serum samples of vaccinated pups (Ingly, 2018). Viruses of rabies (Edries, 1994), canine distemper (Guirguis, 1991), and canine adenovirus (Khodeir et al, 2003) were used (negative control) in IFT. Regarding the SNT results, using of SNAP Parvo test will be useful to minimize the time of evaluation and experimental animals, and also cut the need of professional staff and laboratory facility.

IMMUNOFLUORESCENCE TECHNIQUE (IFT)
Titration and identification of the tested viral vaccines and positive control parvovirus were performed by IF technique, using MDCK cells tissue culture coated microtiter plate as described previously (USDA-APHIS-CVB, 2014). The 50% fluorescent antibody infective dose (FAID50) of the test vaccines and positive control were calculated according to Sperman method (Spearman-Kärber, 1908). This test was used for evaluation of vaccine batches and parvovirus in sensitivity test.
Table 1: Sensitivity of SNAP-Parvo test using standard positive canine parvovirus in comparison with immunofluorescence technique (IFT)

<table>
<thead>
<tr>
<th>Item</th>
<th>Tested dilutions of standard positive canine parvovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>*IFT</td>
<td>-1 -2 -3 -4 -5 -6 -7 -8</td>
</tr>
<tr>
<td>+ve</td>
<td>+ve +ve +ve +ve +ve -ve -ve -ve</td>
</tr>
<tr>
<td><strong>SNAP test (10 strips)</strong></td>
<td>T+ 10 10 10 9 9 0 0 0</td>
</tr>
<tr>
<td>T-</td>
<td>0 0 0 0 0 5 0 0</td>
</tr>
<tr>
<td>F-</td>
<td>0 0 0 1 1 0 0 0</td>
</tr>
<tr>
<td>F+</td>
<td>0 0 0 0 0 5 0 0</td>
</tr>
<tr>
<td>Sensitivity % of SNAP</td>
<td>100 100 100 90 90 0 0 0</td>
</tr>
</tbody>
</table>

* -ve result of IFT refer to normal cells with no detectable virus while +ve refer CPE in tissue culture with detectable virus green spots.
**T+: True Positive, T-: True Negative, F+: False Positive, F-: False Negative

Table 2: Evaluation of the canine parvovirus titer in live attenuated canine parvovirus vaccine batches using immunofluorescence technique (IFT) and SNAP test (rapid ELISA).

<table>
<thead>
<tr>
<th>Vaccine batches No.</th>
<th>IFT +ve titer</th>
<th>SNAP test</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>4.5</td>
<td>5</td>
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<tr>
<td>4</td>
<td>3.5</td>
<td>4</td>
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<tr>
<td>5</td>
<td>3</td>
<td>3</td>
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<tr>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

against canine distemper virus, canine rabies virus and canine adeno virus (negative control samples).

Evaluation of the CPV titer in live attenuated CPV vaccine batches using IFT and SNAP, showed that virus titer was not less than 3 log_{10} in eight vaccine batches as shown in Table-2 and Figure-1.

Evaluation of humoral immune response in the sera of vaccinated puppies was detected by using SNT and the results of the all ten vaccine batches were presented in Table 3. It was showed that, eight batches (1, 2, 3, 4, 5, 7, 9 and 10) reached permissible (protective) limit (1.5 log_{10}) while two batches (6 and 8) did not show protective titer.

DISCUSSION

An effective way to prevent the infection in domestic dogs against CPV infections is vaccination (Greene and Schultz, 2006). Studies on live attenuated CPV vaccines, using limited number of animals, reported increasing antibody titers (Spencer and Burroughs, 1992; van Heerden et al., 2002), which correlates well with protection against CPV disease in domestic dogs (Schultz, 2006).

The present study was aimed to evaluate the existing imported commercial live attenuated CPV vaccine batches in domestic puppies for its efficacy using SNT and tissue culture virus titration using IFA, to develop an alternative indirect potency test instead of IFA tissue culture virus titration with consideration and correlation to SNT. Sensitivity of the rapid SNAP parvo test was evaluated by comparison with the traditional used technique in this study. The rapid SNAP parvo test showed a diagnostic sensitivity of 90% at Cutoff value of 4.5 for detection of CPV compared with IFA tissue culture virus titration. While the specificity showed result 100% when the SNAP parvo test used against canine distemper virus, canine rabies virus and canine adeno virus. These results are in agreement with other workers (Schultz et al., 2008) who conducted sensitivity and specificity of the SNAP® parvo test and reported 100% results for each.

We also tested the imported commercial live attenuated CPV vaccine batches using virus titration on MDCK tissue culture using IFA and SNAP parvo test. The results of virus titration complied with SNAP parvo test indicated the possibility to use SNAP parvo test as alternative.
Table 3: Evaluation of the humoral immune response of vaccinated puppies with live attenuated canine parvovirus vaccine batches using serum neutralization test (SNT).

<table>
<thead>
<tr>
<th>Vaccine Batches No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNT Antibody titer $\text{TCID}_{50}/\text{mL}$</td>
<td>2.1</td>
<td>1.8</td>
<td>2.4</td>
<td>1.95</td>
<td>1.5</td>
<td>1.05</td>
<td>1.65</td>
<td>0.6</td>
<td>1.8</td>
<td>2.25</td>
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</table>

method. The satisfactory vaccine is tested by SNAP parvo test would be having virus titer $10^{0.5}$ greater than the titer calculated by virus titration on tissue culture using IFA with consideration to the permissible (protective) limit of the virus titer which should not less than $10^{3} \text{TCID}_{50}/\text{mL}$ (CFR, 2012).

Evaluation of humoral immune response for the sera of vaccinated puppies was also carried out by SNT to detect neutralizing antibody titer. The results for the ten batches showed that eight batches reached permissible limit of CPV antibodies titer (1.5 log$_{10}$) as suggested by previous workers (Bass et al., 1982; Bonnie et al., 1983), however, two batches (6 and 8) did not show protective titer (Table-3). The SNT was carried out in another study to evaluate the CPV vaccine or CPV hyper immune serum and authors detected neutralizing antibody titer of dogs inoculated by anti-canine parvovirus hyper immune serum as $1024/\text{mL}$ Attyat and Wafaa, (2015). Also Jayalakshmi Vasu et al. (2019) used SNT to compare immune response of two groups of vaccinated puppies, one group given single.

gle booster dose while the other given double booster dose of modified live canine parvovirus vaccine. Similarly, Sherry Glover et al. (2012) applied SNT to detect the humoral immune response of puppies vaccinated with CPV (type 2b) vaccine in comparison with the results obtained from HI technique for the same serum samples. In the current work the results of SNT for vaccinated puppies confirmed and correlated with the results obtained from IP technique and SNAP parvo test which were used in evaluation of the tested vaccine batches.

CONCLUSION

These findings suggest the possibility of using SNAP parvo test for detection of CPV titer in live attenuated CPV vaccine. This rapid ELISA (SNAP test) could be used as a rapid primary test for detection of unsatisfactory vaccines thus may minimize the use of experimental animals, effort and time consumed.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed according to their tasks and approved the final manuscript.

REFERENCES


