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DETECTION OF RETICULOENDOTHELIOSIS VIRUS AS CONTAMINANT OF FOWL POX VACCINES.

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SUMMARY

This study was designated to detect reticuloendotheliosis virus (REV) as a contaminant in fowl pox vaccines. A total of 30 fowl pox vaccine samples were examined for the presence of REV using the *in vitro* and *in vivo* methods. In the in vitro test, the fowl pox vaccine samples were inoculated into chicken embryo fibroblast (CEF) cultures prepared from SPF embryonated chicken eggs and examined by PCR test for detection of REV. In the in vivo test, each fowl pox vaccine sample was inoculated in five days old SPF chicks and kept under observation up to 12 weeks post inoculation (PI); serum samples were collected at 15th, 30th, and 45th days PI for the detection of antibodies against REV by commercial ELISA kit, and tissue samples were collected at 8th and 12th weeks PI for histopathological examination.

Results revealed that: only one imported vaccine sample gave positive results by PCR test a product of 291- bp was obtained by the vaccine sample. Serum samples collected from positive vaccine batch were tested for REV antibodies by ELISA test and the sera were positive. Histopathological examination for liver, spleen and bursa of fabricius revealed the presence of tumor cells in the examined organs and these changes confirmed the results obtained by PCR and ELISA tests

and indicated that the sample is contaminated with REV.

The data clearly indicated that screening of all commercial poultry vaccines to be virus free is an important factor in assuring the biosafety of animal vaccines.

INTRODUCTION:

Pathogens of poultry constitute one of the major problems facing the poultry industry in Egypt causing severe economic losses. For this reason, the vaccines are a major factor in programs to bring the economically important diseases under control. So, they must be safe for the birds, potent and at the same time, free from any biological contaminants either bacterial, fungal or viral.

Some of these vaccines are locally produced and the others are imported. In Egypt, approximately over 90% of the vaccines needed by the poultry industry are imported from different producers in Europe and USA.In the last few years, REV was considered as one of the most important vaccine contaminant (Fadly et al., 1996). Reticuloendotheliosis virus is an avian retrovirus and oncovirus that is antigenically and structurally unrelated to viruses of the leucosis/sarcoma group. Also, it is associated with immunosuppression, runting and neoplasia in domestic poultry and other avian species (Witter, 1991). Dren et al., (1988) observed a chronic neoplasia associated with REV in geese. Also, the most common clinical diseases induced by REV are tumors, immunosuppression and runting disease (Witter and Fadly, 2003). Nicholas and Thornton (1983), Stated that the REV is considered a potential hazard in the use of chicken embryos and cells for preparation of vaccines. Moreover, REV infection persist on the same production sites over period of several years (Bagust, 1993).

REV has been isolated from Marek's disease vaccines (Yuasa et al., 1976). Jackson et al., (1977) reported a case of high mortalities with nervous symptoms and feathering abnormalities (Nakanuke) in chickens vaccinated with MD vaccine at one day old, these case attributed to REV which was detected as vaccine contaminant in one commercial vaccine batch. Bagust and Dennett (1977) isolated REV from commercial MD vaccine (HVT) by serial passages of REV contaminated vaccine on CEF and detected REV antigen using FA test.

In Middle East and Africa, a REV associated lymphoma not related to contaminated vaccine have been reported (Meroz, 1992 and Okoye et al., 1993). On the other hand, in United States Fadly et al., (1996) reported an outbreak of lymphomas in two broiler breeder flocks following using of REV contaminated fowl pox vaccine.

Evidence of the widespread occurrence of REV sequence insertions in fowl pox virus (FPV) genome of field isolates and vaccine strains has increased in the recent years (Diallo et al., 1998 and Garcia et al., 2003).

Recently Singh et al., (2003 and 2005) evidenced that fowl pox virus infection causes lymphoproliferative response in chickens. They also pointed out that an infectious reticuloendotheliosis provirus is present in the majority of the field fowl pox virus genome. Current strains of FPV carrying circulating reticuloendotheliosis virus sequence are becoming more pathogenic to poultry (Tadese et al., 2008).

Fadly and Garcia (2006) reviewed the most common methods used for testing live virus vaccines of poultry for contamination with REV.

The present study aimed to screen our locally produced and imported fowl pox vaccines used in our poultry production in Egypt for the presence of REV through isolation of REV from fowl pox vaccines by inoculation of chicken embryo fibroblast (CEF) and identification using PCR test, inoculation of fowl pox vaccine in SPF chickens, serological detection of antibody against REV in inoculated SPF chickens using ELISA test and histopathological examination of SPF chickens inoculated with REV isolate.

MATERIAL AND METHODS:

Vaccine Samples:

Different batches of fowl pox vaccines either imported or locally produced were examined for detection of REV as a contaminant. A total of 30 fowl pox vaccine batches (4 batches are locally produced and 26 batches are imported) were examined in this study. Each vaccine batch was represented by 6 vials: two were pooled and propagated on CEF culture then examined by PCR and two were pooled and inoculated in SPF chicks, where the other two vials were kept at +4C for eventual need.

Specific pathogen free (SPF) fertile eggs and chickens:

A total of 250 SPF fertile chicken eggs were obtained from SPF farm, Egypt. Twenty chicken embryos 9-11days old were used for preparation of CEF culture and the others were kept in the incubator until hatching. A total of 200, five day-old SPF chickens were used for experimental infection.

Media and solutions for tissue culture:

Tissue culture media:

Dehydrated Eagl's minimum essential medium (MEM), Sigma, USA, with Eagl's salts and L-glutamine without sodium bicarbonate was used. It was reconstituted in double distilled water, pH was adjusted to 7.2 and finally sterilized by filtration through 0.95 millipore membrane filter. Inactivated fetal calf serum was added in concentration of 5% for growth medium and 2% in maintenance medium. Antibiotics (penicillin 1000IU and streptomycin 500 µg)were added at 1 ml stock / 100 ml medium.

Testing of vaccines for REV contamination by tissue culture inoculation:

Preparation of the inoculum:

The preparation of fowl pox vaccine sample for detection of REV contamination using tissue culture was carried out according to Fadly et al. (1996) as following:

The fowl pox vaccine sample was reconstituted in 10 ml MEM then ten fold serial dilution was carried out until reach the dilution containing 10 bird dose/ml.

Inoculation of the prepared inocula:

The prepared vaccines were inoculated on drained secondary CEF cultures planted in 6 well tissue culture plates and incubated for 30 minutes at 37° C for adsorption, negative cells control represented by one uninoculated well, maintenance medium (with 2% calf serum) was added to all wells and the plates were incubated at 37° C in 5% CO2 incubator for 4 days with daily observation.

Harvesting the inoculated cultures:

Four days post inoculation, the plates were subjected to 3 cycles of freezing and thawing then the cell suspensions were harvested in small vials and kept at -70° C for detection of REV by polymerase chain reaction.

Polymerase chain reaction for REV detection:

Extraction of fowl pox genome:

1.Commercial kits were used for extraction of fowl pox genome, and the procedures

were recommended by the manufacturers (Biobasic Company) as following:

2.0.5 ml of the sample was harvested in 2.0 ml collection tube then centrifugation at 3000 rpm for 3 minutes at 4C. Discard the supernatant.

3.Add 0.8 ml TBP buffer to the collection tube, vortex gently then centrifuged at 3000 rpm for 3 minutes. Discard supernatant and repeat.Add 0.5 ml TBM buffer to the collection tube vortex the tube vigorously and then 3 µl proteinase-K. Incubate at 55° C for 30 minutes.

4.If insoluble material is visible, centrifuge for 2 minutes at 5,000 rpm. Save the supernatant to 2.0 ml collection tube, then add 360 µl absolute ethanol.

5.Apply the mixture to EZ-10 column that is in a 2.0 ml collection tube. Centrifuge at 8,000 rpm for 1 minute, discard the flow through in the collection tube.

6.Add 500 µl of wash solution, and centrifuge at 8,000 rpm for 1 minute and repeat.

7.Discard the flow through centrifuge at 8,000 rpm for 1 minute to remove residual amount of wash solution.

8.Place the column into clean 1.5 ml microfuge tube. Add 30-50 µl elution buffer into the center part of membrane in the column. Incubate the tube at 37 or 50C for 3 minutes.

9.Centrifuge at 10,000 rpm for 1 minute to elute DNA from the column.

Polymerase chain reaction test: Commercial kits were used in PCR test for detection of REV and the procedures were recommended by the producer (Fermentas Company) as following:

Add in a thin walled PCR tube on ice

1. 25 µl of 2x PCR master mix.
2. 0.1-1.0 µm forward primer.
3. 0.1-1.0 µm reverse primer.
4. 10 pg – 1 Mg template DNA.
5. Water, nuclease-free up to 50 µl.

6.Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of the tube.

7.Overlay the sample with mineral oil or add an appropriate amount of wax.

8.Place the samples in a thermocycler and start the program.

9.Cycling parameters carried out as recommended by Aly et al., (1993) were 94° C for 2 minutes for initial denaturation followed by 39 cycle at 94° C for 1 minute,55° C for 2 minutes and 72° C for 1 minutes. A final elongation proceeded at 72° C for 6 minutes.

Experiment (1):

Testing of vaccines for REV contamination by chickens inoculation:

Preparation of the inoculum:

The preparation of fowl pox vaccine sample for detection of REV contamination using chickens was carried out according to Fadly and Witter, (1997) where the fowl pox vaccine sample was reconstituted in MEM at the rate of 100 doses/ml.

Inoculation of chickens and blood collection:

The diluted vaccine samples were inoculated into five day old SPF chicks. Each sample was inoculated in five SPF chicks by subcutaneous route with 0.2 ml of the inoculum per chick, and kept in an isolator for 45 days post inoculation. Negative control group of chicks was inoculated S/C with sterile saline and kept parallel to the inoculated group. After 30 and 45 days post inoculation, blood samples were collected from surviving birds, sera separated and inactivated by heating at 56° C for 30 minutes then kept at -20° C until tested for REV antibodies by ELISA test.

Detection of REV antibodies by ELISA:

Commercial ELISA kits (IDEXX Laboratories) were used for detection of REV antibodies in the sera of SPF chickens at 30 and 45 days post inoculation. The procedure of the test was carried out according to the manufacturers.

Experiment (II):

In vivo assay for detection of REV Fowl pox vaccine no.22 which gave positive results in PCR and ELISA tests was retested in vivo. Prior to chicks inoculation, vaccine sample was reconstituted in MEM at a rate of 10 ml/1000 doses. Fifteen ; 5 day old SPF chicks were used in this experiment, 10 chicks were inoculated subcutaneously with 0.2 ml / bird (100 doses of fowl pox vaccine) of the prepared vaccine, and 5 chicks were inoculated with saline and kept as negative control.

All chicks were kept up to 12 weeks post inoculation, serum samples were obtained individually at 15th, 30th and 45th days post inoculation and tested by ELISA for detection of antibodies against REV using commercial ELISA kit.

At the end of 8th and 12th week post inoculation 5 birds each time were sacrificed and tissue specimens were collected from liver, spleen and bursa of Fabricious for histopathological examination.

Histopathological examination:

The properly collected tissue specimens from the liver, spleen and bursa

of Fabricius were immediately fixed in 10% neutral buffered formalin solution for at least 24 hours. The fixed specimens were washed in tap water and then processed in ascending grades of ethyl alcohol for dehydration, cleared in xylon and paraffin embedded. Specimens were dehydrated in alcohol, cleared in xylol and embedded in paraffin. Sections of 4 -5 μ thick were obtained and then stained with Harris hematoxylin and eosin (H & E), mounted in Canada balsam, dried and examined with light microscope (Banchroft, J. D. and Stevens, A., (1996).

RESULTS

Testing of vaccine for REV contamination by tissue culture inoculation:

The inoculated SPF cultures were daily observed for 4 days and then the cultures were subjected to 3 cycles of freezing and thawing then the cell suspensions were harvested in small vials and kept at -70° C.

PCR test was carried out for detection of REV antigen in vaccine inoculated onto CEF cultures. As shown in figure (1), a product of 291-bp was obtained by vaccine sample no. 22, while other vaccine samples gave negative results.

Testing of vaccines by chicks inoculation:

The inoculated birds were kept under daily observation for 7 weeks post inoculation and blood samples were collected at both 30 and 45 days post inoculation and sera were separated.

ELISA test using commercial ELISA kits was used for detection of REV antibodies in the sera of SPF chickens previously inoculated with fowl pox vaccine samples.

Data presented in table 1, 2 and 3 are showing that one inoculated group of chickens (vaccine no, 22) was positive for REV antibodies at both 30 and 45 days post inoculation, while 29 out of 30 vaccines inoculated chicks groups were negative for REV antibodies at both 30 and 45 days post inoculation.

Histopathological examination:

Liver: Hispathological examination of the liver revealed marked atrophy of hepatocytes as a result of pronounced congestion of hepatic sinusoids after 8 and 12 weeks post inoculation. Small foci of lymphoblast cells infiltration were detected after 12 weeks post inoculation (Fig. 2, 3)

Spleen: Histopathologic examination of the spleen 8 weeks after inoculation revealed ill-defined multiple focal proliferation of lympho-histiocytic cells with hyperchromatic nuclei and moderate to abundant slightly basophilic cytoplasms. The cells have a close relation to arterioles in distribution (Fig 4). After 12 weeks the spleen disclosed defuse focal investment with lympho-histiocytes cells. The cells showed pronounced pleomorphis, ranged from small rounded, ovoid and large lymphoblast cells, with vesicular nucleus. Some elongated and degenerated cells were also observed. The neoplastic cells had large round to oval vesicular nuclei with prominent chromatin clumps adjacent to the inner surface of the nuclear envelope (Fig. 5, 6).

Bursa of Fabricious: 8 weeks post inoculation the bursa of fabricious showed mild ill defined aggregations of lymphoid cells difficult to distinguish from normal bursal lymphoid cells. The cells were inter and intrafolicullary distributed (Fig. 7,8). At 12 weeks post inoculation the bursa showed marked atrophy with moderate inter and intra follicular prolifertation of lymphoid cells (lymphoreticular cells) (Fig. 9, 10).

The cells have the same morphology as described in spleen and many of them showed degeneration.

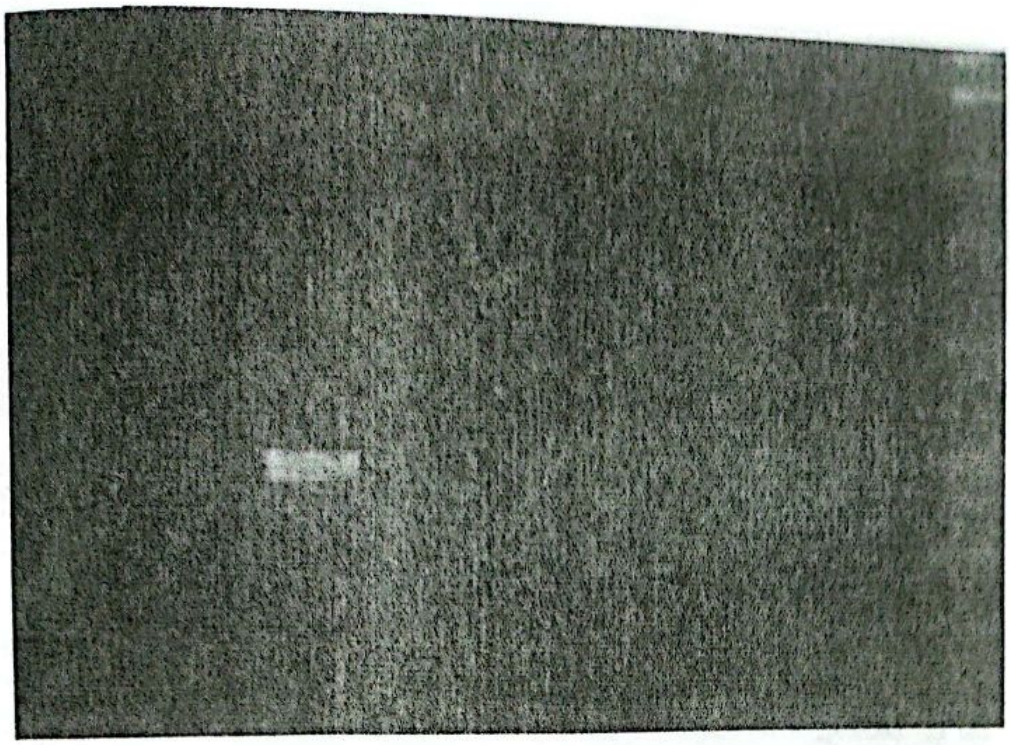

Fig. (1): Detection of proviral REV sequences in DNA of different fowl pox vaccine samples. A fragment of 291 bp was detected in lane (3)

Lane 1: Negative control cell
Lane 2: Sample No. (21)
Lane 3: Sample No. (22)
Lane 4: Sample No. (23)
Lane 5: Sample No. (24)
Lane 6: Sample No. (25)
Lane 7: Sample No. (26)
Lane 8: Molecular size marker

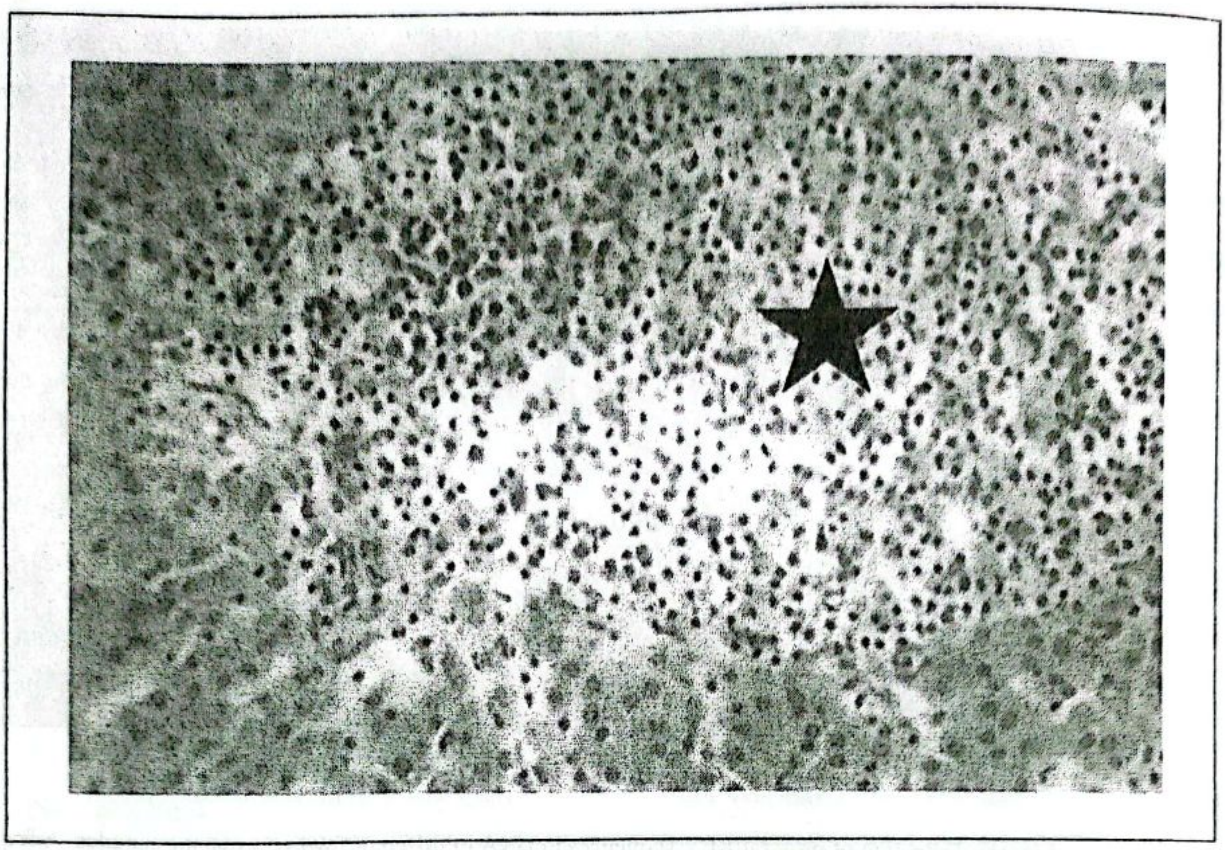

Fig. (2): Liver, 8 weeks post infection showing large area of extensive infiltrations and aggregations of the hyperplastic mononuclear cell (asterisk) in betweeb the severely degenerated and necrotic hepatocytic cells (H & E x400)

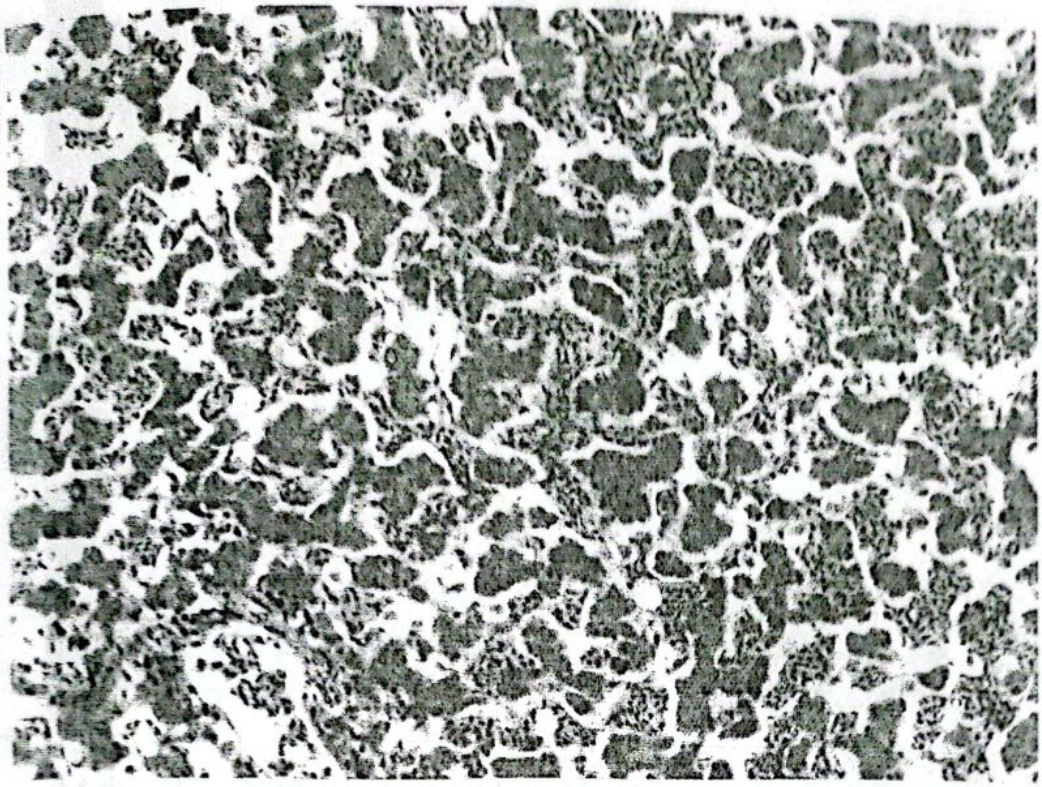

Fig. (3): Liver, 12 weeks post infection showing marked atrophy of hepatic acinai as well as pronounced congestion of hepatic sinusoids (H & E x200)

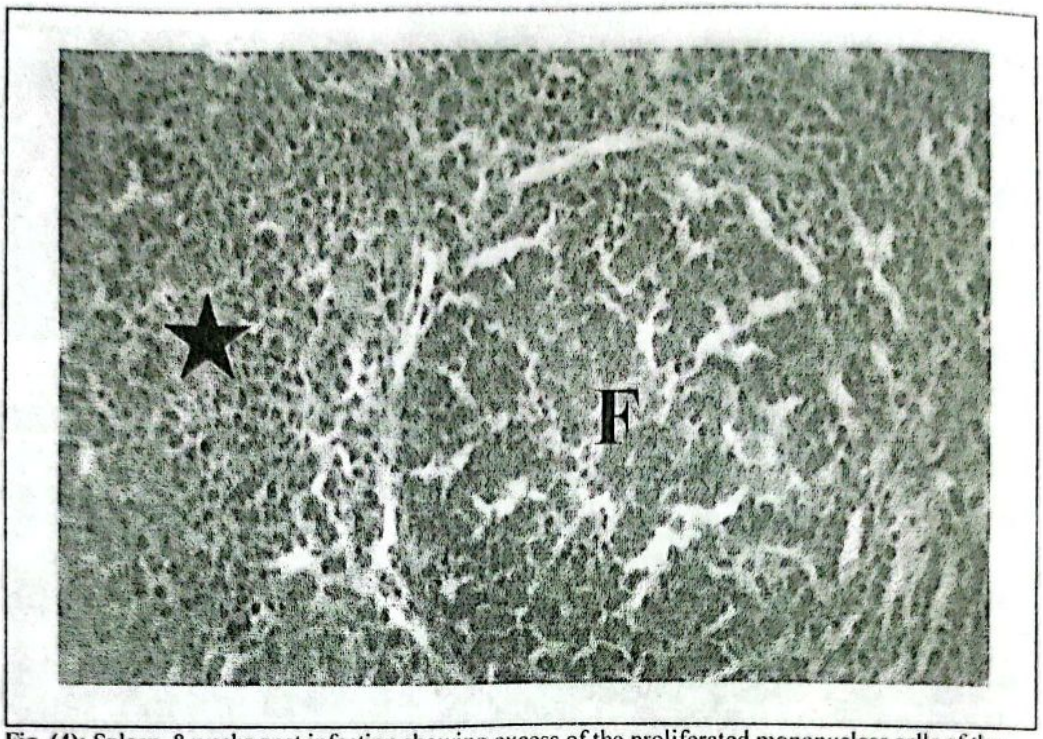

Fig. (4): Spleen, 8 weeks post infection showing excess of the proliferated mononuclear cells of the reticuloendothelial elements (asterisk) one large hypertrophied lymph follicle (F) (H & E x400)

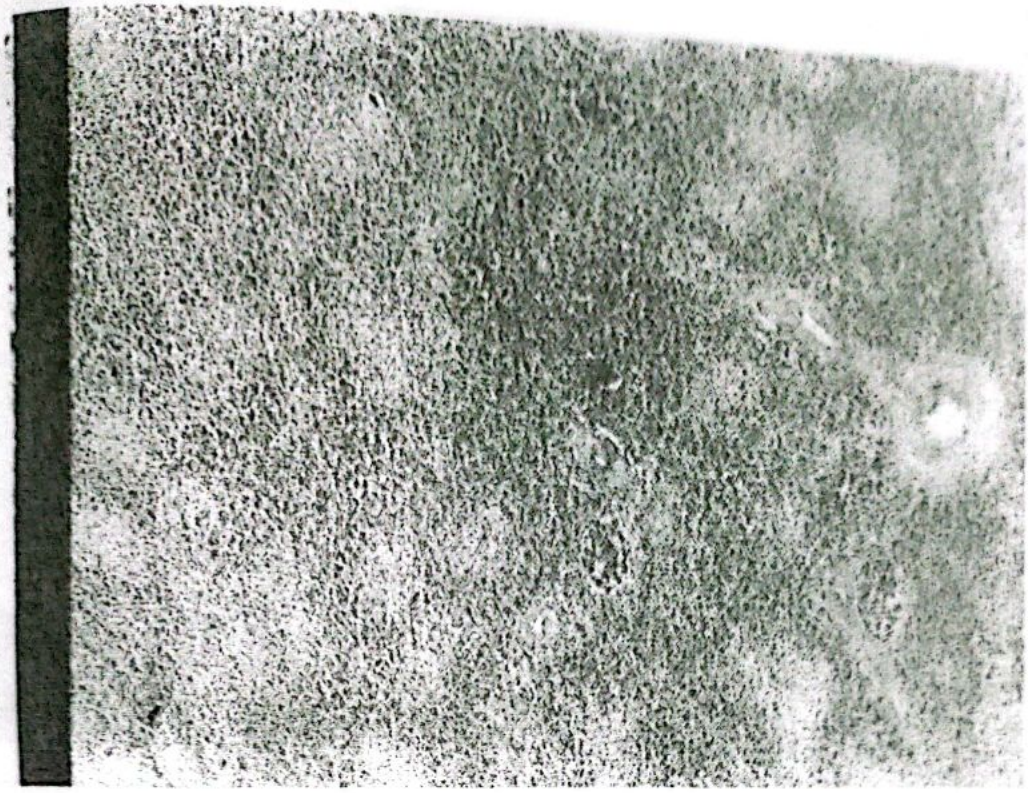

Fig. (5): Spleen, 12 weeks post infection showing diffuse focal proliferation of lymphocytes-histocytes cells (H & E x50)

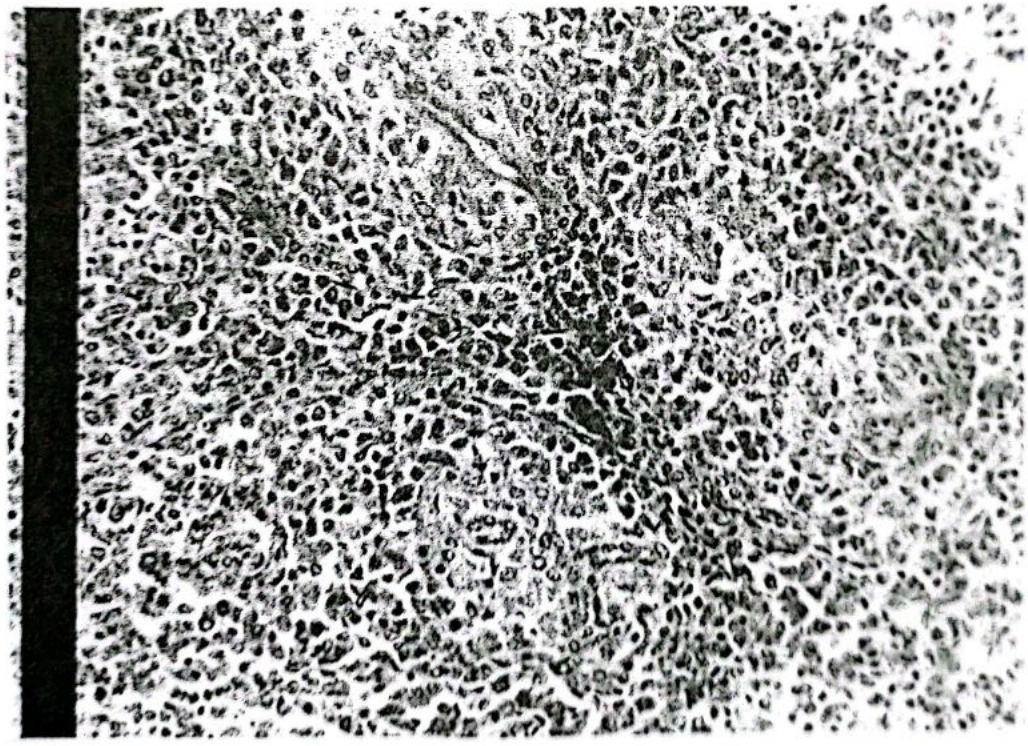

Fig. (6): Spleen, 12 weeks post infection showing moderate magnification of Fig. (7), notice vesicular nucleus and margination of chromatin (H & E x200)

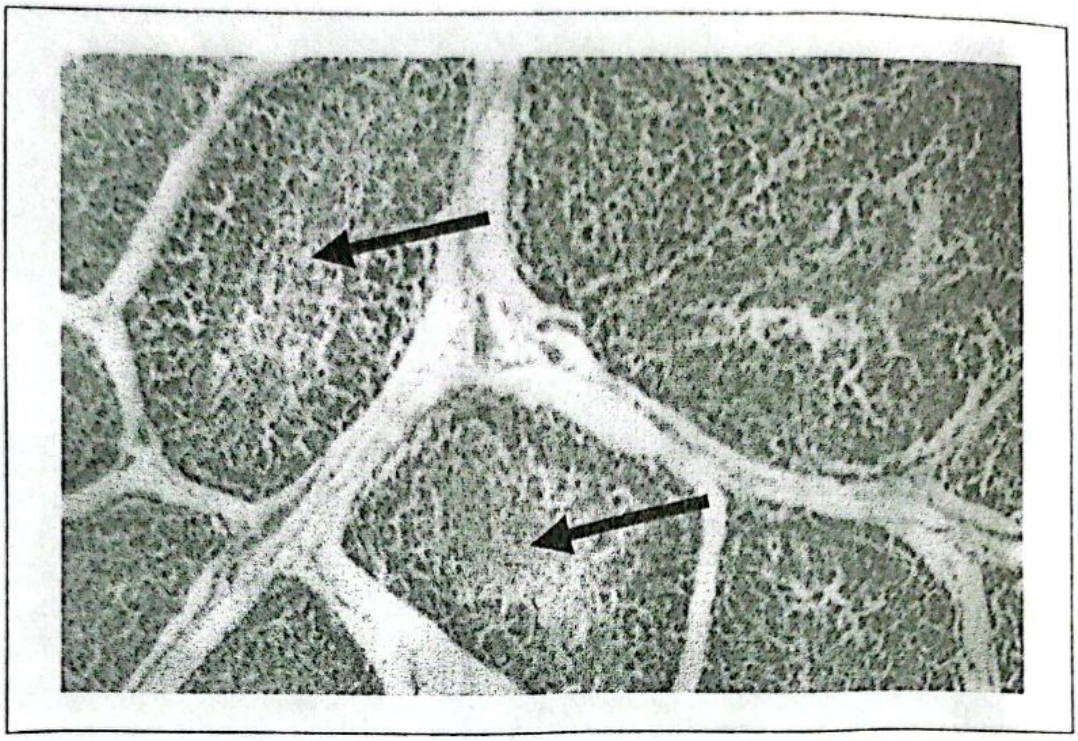

Fig. (7): Bursa of Fabricius, 8 weeks post infection showing diffuse hyperplastic changes in most of all the lymph follicles (arrows) (H & E x250)

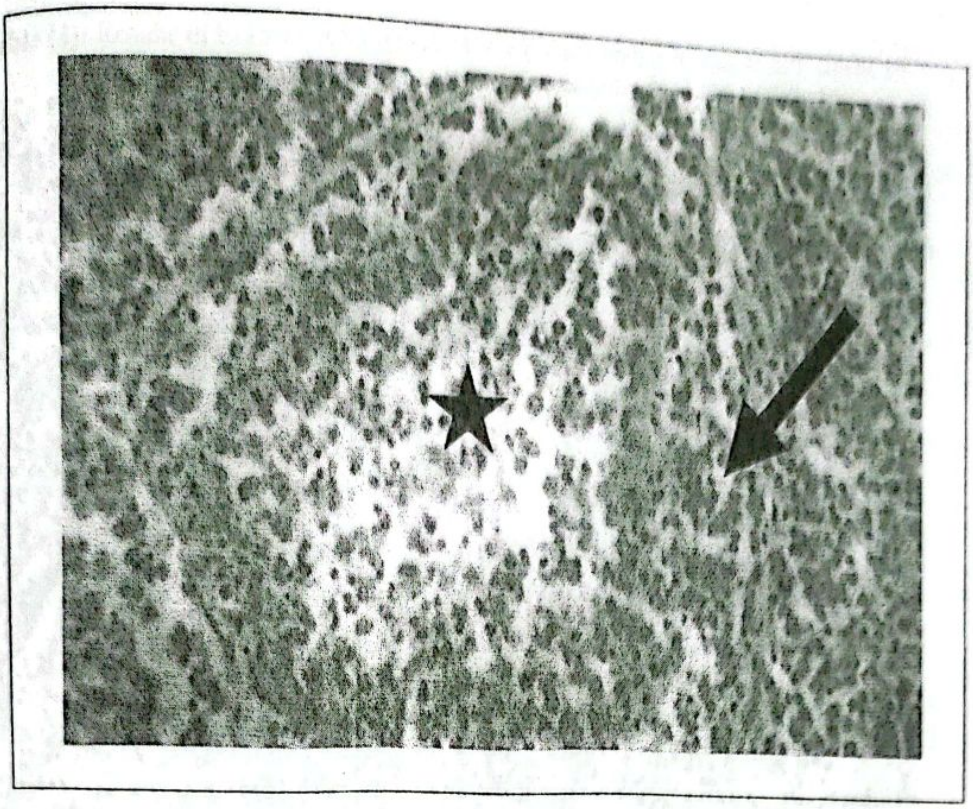

Fig. (8): Bursa of Fabricius, 8 weeks post infection showing damaged follicle with central lymphocytic necrosis (asterisk) and peripheral infiltration and aggregations of mononuclear cells (arrow) (H & E x400)

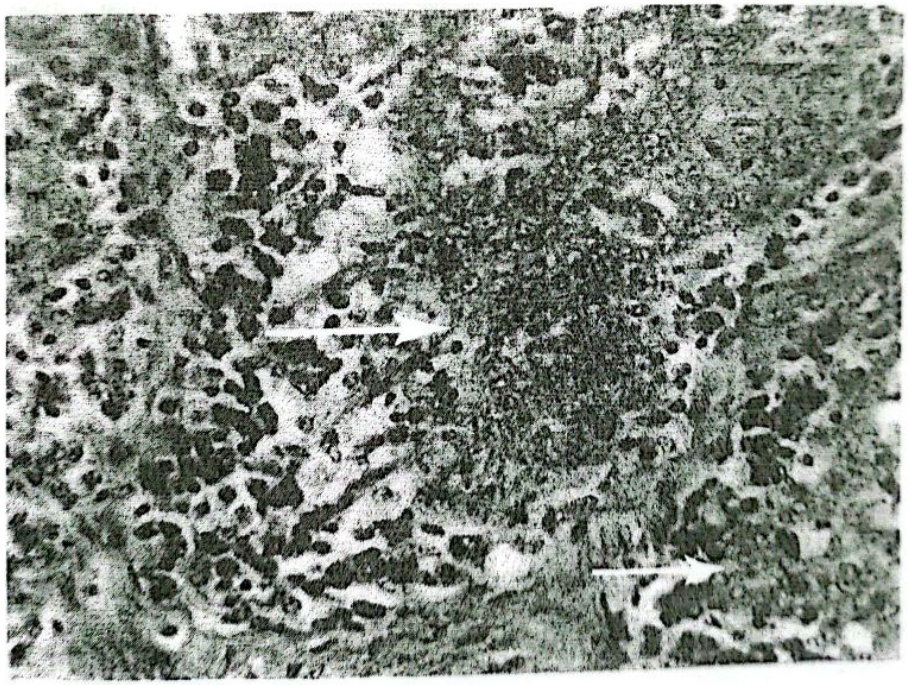

Fig. (9): Bursa of Fabricius, 12 weeks post infection showing inter & intra follicular aggregation of lymphocytes histocytes cells (H & E x400)

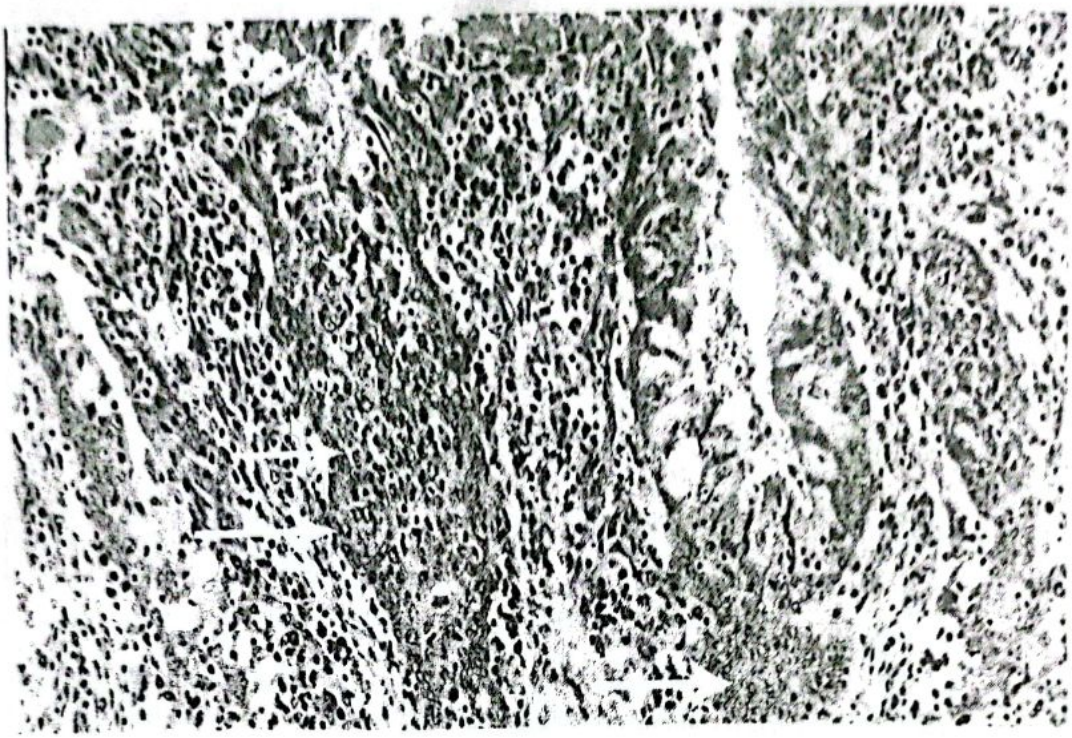

Fig. (10): Bursa of Fabricius, 12 weeks post infection showing inter & intra follicular aggregations of lymphocytes histocytes cells (notice atrophy of the follicles) (H & E x200)

Table (1): Results of ELISA test for detection of REV antibodies in sera of SPF chicks 30 days post inoculation with fowl pox vaccine samples from No.1 to No.10

Chick group n=5	Mean of optical density	*S/P ratio	No. positive/no. examined	Interpretation
1	0.048	0.012	0/5	Negative
2	0.0485	0.015	0/5	Negative
3	0.05	0.017	0/5	Negative
4	0.052	0.101	0/5	Negative
5	0.055	0.024	0/5	Negative
6	0.057	0.027	0/5	Negative
7	0.053	0.022	0/5	Negative
8	0.051	0.019	0/5	Negative
9	0.061	0.034	0/5	Negative
10	0.059	0.03	0/5	Negative
uninoculated chicks	0.074	0.052	0/5	Negative

- Negative control mean (NCM) = 0.038
- Positive control mean (PCM) = 0.7
- Corrected positive control (CPC) = 0.662

* S/P ratio more than 0.5 was considered positive for REV antibodies.

Continue Table (1): Results of ELISA test for detection of REV antibodies in sera of SPF chicks 30 days post inoculation with fowl pox vaccine samples from No. 11 to No. 20

Chick group n = 5	Mean of optical density	* S/P ratio	No. positive/No. examined	Interpretation
11	0.04	0.005	0/5	Negative
12	0.039	0.003	0/5	Negative
13	0.04	-0.001	0/5	Negative
14	0.04	0.001	0/5	Negative
15	0.036	-0.003	0/5	Negative
16	0.037	-0.002	0/5	Negative
17	0.038	0.003	0/5	Negative
18	0.038	0.008	0/5	Negative
19	0.037	-0.0002	0/5	Negative
20	0.038	0.0005	0/5	Negative
uninoculated chicks	0.037	-0.0022	0/5	Negative

- Negative control mean (NCM) = 0.038
- Positive control mean (PCM) = 0.485
- Corrected positive control (CPC) = 0.444

* S/P ratio more than 0.5 was considered positive for REV antibodies.

Continue Table (1): Results of ELISA test for detection of REV antibodies in sera of SPF chicks 30 days post inoculation with fowl pox vaccine samples from No. 21 to No. 30

Chick group n=5	Mean of optical density	*S/P ratio	No. positive/no. examined	Interpretation
21	0.086	0.177	0/5	Negative
22	0.202	0.6	5/5	positive
23	0.0812	0.161	0/5	Negative
24	0.079	0.15	0/5	Negative
25	0.087	0.18	0/5	Negative
26	0.075	0.14	0/5	Negative
27	0.083	0.16	0/5	Negative
28	0.088	0.18	0/5	Negative
29	0.088	0.19	0/5	Negative
30	0.063	0.09	0/5	Negative
uninoculated chicks	0.04	0.006	0/5	Negative

- Negative control mean (NCM) = 0.039
- Positive control mean (PCM) = 0.3
- Corrected positive control (CPC) = 0.26

* S/P ratio more than 0.5 was considered positive for REV antibodies.

Table (2): Results of ELISA test for detection of REV antibodies in sera of SPF chicks 45 days post inoculation with fowl pox vaccine samples from No. 1 to No. 10

Chick group n=5	Mean of optical density	*S/P ratio	No. positive/no. examined	Interpretation
1	0.0305	0.063	0/5	Negative
2	0.0605	0.017	0/5	Negative
3	0.06	0.016	0/5	Negative
4	0.058	0.012	0/5	Negative
5	0.069	0.036	0/5	Negative
6	0.091	0.081	0/5	Negative
7	0.054	0.005	0/5	Negative
8	0.054	0.004	0/5	Negative
9	0.056	0.009	0/5	Negative
10	0.055	0.007	0/5	Negative
uninoculated chicks	0.065	0.02	0/5	Negative

- Negative control mean (NCM) = 0.052
- Positive control mean (PCM) = 0.53
- Corrected positive control (CPC) = 0.48

* S/P ratio more than 0.5 was considered positive for REV antibodies.

Continue Table (2): Results of ELISA test for detection of REV antibodies in sera of SPF chicks 45 days post inoculation with fowl pox vaccine samples from No. 11 to No. 20

Chick group n=5	Mean of optical density	*S/P ratio	No. positive/no. examined	Interpretation
11	0.038	-0.0013	0/5	Negative
12	0.036	-0.006	0/5	Negative
13	0.037	-0.003	0/5	Negative
14	0.039	0.0013	0/5	Negative
15	0.036	-0.007	0/5	Negative
16	0.034	-0.011	0/5	Negative
17	0.035	-0.01	0/5	Negative
18	0.042	0.009	0/5	Negative
19	0.037	-0.003	0/5	Negative
20	0.036	-0.006	0/5	Negative
uninoculated chicks	0.035	-0.01	0/5	Negative

- Negative control mean (NCM) = 0.039
- Positive control mean (PCM) = 0.42
- Corrected positive control (CPC) = 0.38

* S/P ratio more than 0.5 was considered positive for REV antibodies.

Continue Table (2): Results of ELISA test for detection of REV antibodies in sera of SPF chicks 45 days post inoculation with fowl pox vaccine samples from No. 21 to No. 30

Chick group n=5	Mean of optical density	*S/P ratio	No. positive/no. examined	Interpretation
21	0.049	0.002	0/5	Negative
22	0.635	1.27	5/5	positive
23	0.047	-0.002	0/5	Negative
24	0.047	-0.001	0/5	Negative
25	0.048	0.0	0/5	Negative
26	0.07	0.047	0/5	Negative
27	0.05	0.004	0/5	Negative
28	0.047	-0.002	0/5	Negative
29	0.05	0.004	0/5	Negative
30	0.048	0.001	0/5	Negative
uninoculated chicks	0.046	-0.005	0/5	Negative

- Negative control mean (NCM) = 0.048
- Positive control mean (PCM) = 0.51
- Corrected positive control (CPC) = 0.46

* S/P ratio more than 0.5 was considered positive for REV antibodies.

Table (3): Results of ELISA for detection of antibodies in serum samples from SPF chickens inoculated with pox vaccine sample No. 22

	15th day post inoculation			30th day post inoculation			45th day post inoculation		
	Mean of absorbance	* S/P ratio	Interpre-tation	Mean of absorbance	* S/P ratio	Interpre-tation	Mean of absorbance	* S/P ratio	Interpre-tation
Inoculated chicks	0.22	0.53	Positive	0.64	1.28	Positive	0.81	1.46	positive
	0.26	0.66	Positive	0.55	1.1	Positive	0.82	1.47	Positive
	0.23	0.57	Positive	0.49	0.97	Positive	0.77	1.38	Positive
	0.25	0.62	Positive	0.60	1.18	Positive	0.97	1.7	Positive
	0.31	0.83	Positive	0.63	1.26	Positive	0.88	1.6	Positive
	0.28	0.71	Positive	0.70	1.42	Positive	0.73	1.3	Positive
	0.23	0.56	Positive	0.74	1.5	Positive	0.80	1.44	Positive
	0.24	0.61	Positive	0.63	1.27	Positive	0.86	1.5	Positive
	0.24	0.60	Positive	0.54	1.06	Positive	0.70	1.26	Positive
	0.27	0.71	Positive	0.57	1.13	Positive	0.84	1.5	Positive
Overall mean me mean	0.25	0.64		0.61	1.2		0.82	1.45	
▪ Uninoculated chicks(control)	0.043	-0.02	Negative	0.057	0.002	Negative	0.06	-0.005	Negative

▪ Control data is overall mean of 5 uninoculated chicks.

* S/P ratio more than 0.5 was considered positive for REV antibodies.

- Over all mean of the S/P ratio of the sample 15th days post inoculation =0.64
- Over all mean of the S/P ratio of the sample 30th days PI = 1.2
- Over all mean of the S/P ratio of the sample 45th days PI = 1.45

At 15th day post inoculation:
- Negative control mean (NCM) = 0.05
- Positive control mean (PCM) = 0.37
- Corrected positive control (CPC) = 0.32

At 30th day post inoculation:
- Negative control mean (NCM) = 0.056
- Positive control mean (PCM) = 0.51
- Corrected positive control (CPC) = 0.45

At 45th day post inoculation:
- Negative control mean (NCM) = 0.063
- Positive control mean (PCM) = 0.58
- Corrected positive control (CPC) = 0.52

DISCUSSION

In the present study, thirty batches of fowl pox virus vaccines collected from different sources (locally and imported vaccines) were screened for the detection of REV as contaminant. Vaccines were tested by two assays, the in vitro assay using CEF cultures then PCR for detection of REV equence using the 5` LTR primers, and the in vivo assay by inoculation of fowl pox vaccines in five days old SPF chicks, detection of REV antibodies by ELISA test and histopathological examination of the internal organs.

Results of the in vitro assay revealed that one batch of FPV vaccines out of thirty (3.33%) was positive in PCR test. A product of 291bp were obtained, as reported by Witter and Fadly (2003), who detected the proviral DNA by PCR assays that amplify the 291 bp product of REV LTR has been shown to be a sensitive and specific method for detection of various strains of REV in CEF cultures as well as in blood and tumors of infected chickens. The percentage of positive vaccines (3.33%) is relatively low when compared to that obtained by Garcia et al.(2003) who examined field isolates and vaccines of fowl pox virus for detection of REV by PCR using two types of primers: the 5'LTR primer and env. sequence primer. They found that all field isolates gave positive results with both primers while 50% of the examined vaccinal strains were amplified only with REV 5` LTR primer but not with the env. sequence primer. These results were also related to the obtained results by Diallo et al.(1998); Singh et al. (2000), Kim and Tripathy (2001) when they found that none of the examined vaccinal strains contain env. sequence while 12 of 17 tested vaccines were positive for REV when they used the 5` LTR primer. The difference in percentage of positive vaccines in these reports and our study may be due to that the tested FPV vaccines were produced by different manufactures and imported from many countries and it may not exactly contain the same strain in each time. Also, may be due to that we used one primer (5`LTR) while other studies used two primers (the 5` LTR and env. sequence primer). However, Hertig et al. (1997) reported that PCR amplification was obtained for one of the four vaccine strains with REV env. sequence. The results of in vivo assay are correlated with that of in vitro assay.

Antibodies against REV were detected in one group of chickens (the group which inoculated by pox vaccine sample No. (22)) and gave positive result by PCR. REV antibodies were firstly detected at 15th day post inoculation using ELISA test and this result agreed with that reported by Motha MXJ. (1984) who detected REV antibodies in inoculated chickens as early as 16 days post inoculation. However, Aly et al., (1993) detected REV antibodies at 3 weeks post inoculation and not before. On the other hand, Fadly and Witter, (1997) failed to detect REV antibodies in SPF chickens inoculated at 5 days old with REV

contaminated fowl pox vaccine neither at 3 nor 4 weeks post inoculation using the immuno-fluorescence (IF) assay. This discrepancy in results of Fadly and Witter, (1997) and that obtained by other workers may arise from that Fadly and Witter, (1997) used a contaminated fowl pox vaccine as inoculum while Motha , (1984) and Aly et al., (1993) used a pure REV preparations. These results suggesting that the dose of RE virus in the inoculum may play role in the response of chickens and also the presence of other agents (fowl pox virus in our study) may interfere with the response of inoculated chickens to produce antibodies against REV. Another factor could attributed to that the detection of REV antibodies in the present study was carried out using ELISA test while other researchers used the IF assay. These results indicated that, the ELISA test has shown to be most sensitive and specific method for detection of REV antibodies which supported by Smith and Witter (1983).

In this study a specific REV neoplastic lesions in liver, spleen and bursa were observed at 8^{th} and 12^{th} week post inoculation, and this agrees with results obtained by (Bohls et al., 2006) who detect neoplasia at 8th week post inoculation of prairie chickens with REV.

Finally, we can conclude that contamination of vaccine, partial or complete insertion of REV genome in other viruses and developing new control method represent important challenge that must be addressed in order to develop effective strategies for control of REV infection in poultry and for obtaining maximum vaccination results.

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دراسة عن الكشف عن فيروس الرتيكلوأندوثيليوزيس كملوث فيروسى فى لقاح جدرى الطيور

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استهدفت هذه الدراسة الكشف عن فيروس الرتيكلوأندوثيليوزيس كملوث فيروسى فى لقاح جدرى الطيور سواء أكان مستوردا أو منتج محليا فى هذه الدراسة تم اختبار عدد 30 عينة من لقاح جدرى الطيور (26 عينة مستوردة من شركات مختلفة، 4 عينات منتجة محليا) وتم فحص هذه العينات بطريقتين :الاولى فى المعمل in vitro والثانية فى الطائر in vivo.

أولا الاختبار فى المعمل in vitro:

حيث تم حقن اللقاحات على مزارع نسيجية CEF محضرة من بيض خالى من المسببات المرضية SPFوتم حفظ هذه المزارع النسيجية فى الحضانة عند درجة حرارة 37° مئوية لمدة 4 أيام مع الفحص اليومى .ثم تم عمل اختبار تفاعل البلمرة المتسلسل PCR للكشف عن وجود فيروس الرتيكلوأندوثيليوزيس.

ثانيا الاختبار فى الطائر in vivo:

كذلك تم حقن كل عينة من لقاح جدرى الطيور فى 5 كتاكيت خالية من المسببات المرضية SPFعمر 5 أيام وتم الاحتفاظ بهذه الكتاكيت لمدة 7 أسابيع بعد الحقن. تم تجميع سيروم من هذه الكتاكيت عند 30،45 يوم بعد الحقن وتم عمل اختبار الاليزا باستخدام Commercial kitللكشف عن وجود أجسام مضادة لفيروس الرتيكلوأندوثيليوزيس

وكانت النتائج كالتالى:

أولا بالنسبة للاختبار فى المعمل in vitro:

باستخدام PCR فقد وجد أن 29 عينة (96.6%) خالية من التلوث بفيروس الرتيكلوأندوثيليوزيس بينما كانت عينة واحدة فقط (3.33%) ملوثة.

ثانيا بالنسبة للاختبار فى الطائر in vivo:

وجد أن نفس العينة التى اعطت نتائج ايجابية فى الكشف المعملى باستخدام PCR قد أعطت نتائج ايجابية باستخدام الاليزا فقد تم اكتشاف أجسام مضادة لفيروس الرتيكلوأندوثيليوزيس فى الكتاكيت المحقونة لهذا اللقاح عند 30،45 يوم بعد الحقن.

تم اعادة اختبار العينة الايجابية وذلك بحقنها مرة ثانية فى كتاكيت خالية من المسببات المرضية وتم متابعة هذه الكتاكيت لمدة 12 أسبوع بعد الحقن وتم تجميع عينات سيروم للكشف عن وجود أجسام مضادة لفيروس الرتيكلوأندوثيليوزيس عند عمر30،45،15 يوم بعد الحقن وكانت النتيجة ايجابية فى المرات الثلاثة .كذلك تم ذبح بعض الدجاج عند عمر 8،12 أسبوع من الحقن وتم تجميع الكبد والطحال وغدة فابريشيوس وذلك لعمل اختبار فحص هستوباثولوجى وكانت نتيجة هذا الاختبار ايجابية بوجود خلايا سرطانية فى هذه الاعضاء ايضا مما يؤكد تلوث هذا اللقاح بفيروس الرتيكلوأندوثيليوزيس.

يلخص من هذه الرسالة انه لابد ان يتلقى لفيروس الرتيكلوأندوثيليوزيس بعض الاهتمام فى الكشف عنه فى جميع اللقاحات سواء كانت محلية أومستوردة وكذلك تطوير الطرق المستخدمة فى الكشف عن هذا الفيروس لما له من اهمية اقتصادية فى مجال الثروة الداجنة سواء عن طريق تقليل استجابة الجهاز المناعى للطائر للقاحات المختلفة أو تقليل الكفاءة الانتاجية للطيور المصابة.