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Research Article
Histopathological and Serological Diagnosis of Avian Reticuloendotheliosis in Cross-bred Chicken Farms in Delta Egypt

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Abstract

Background: The reticuloendotheliosis virus (REV) in chicken induces a variety of deleterious effects, tumors and increased mortalities causing considerable economic losses. In this study the reticuloendotheliosis infection status were performed through antibody determination and histopathological findings in chickens in delta Egypt. **Materials and Methods:** Fourteen cross-bred chicken flocks varied from 12-25 weeks age and reared in two provinces in delta Egypt were investigated. About 691 birds were submitted for pathological investigations. Two hundred and seventy one serum samples were collected for serology. Further 20 samples from seropositive and seronegative birds were used for hematological screening. **Results:** In total of 691 birds 278 birds (40.2%) unusual tumor formation in the head and neck regions and enlarged liver, spleen and kidneys. The features of common pathologic lesions were shown as multiple neoplastic nodules of homogeneous lymphocytes in the livers and spleens. Histopathology of the head region revealed hypercellularity with intercellular transudate, focal aggregation of inflammatory cells around the diluted blood vessels and hemorrhages in tumor mass. Visceral lymphomas occurred in a 25 weeks old chicken flock. The serological prevalence for the REV antibody ranged from 25.0-100.0% at 12th and 25th weeks of age, respectively. The total WBCs, heterophils and monocytes count were significant higher in birds with tumor. **Conclusion:** The present study describes the first report for prevalence of REV in cross-bred chicken in delta region in Egypt. The serological results suggested circulation of REV within chicken in delta Egypt. Further study is paramount for improving the control measures for REV in chickens.

Key words: Reticuloendotheliosis, chickens, ELISA, pathology, hematology

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reticuloendotheliosis viruses (REV) are a group of avian retroviruses that infect turkeys, ducks, quails and chickens causing marked immunosuppression and disease which is sporadically neoplastic[1,2]. The virus is widespread and the infection can be transmitted by vertical or horizontal means. The horizontal transmission takes place by direct and indirect contact. The virus could be detected in feces, cloacal swabs and litter and could be disseminated by needles used for administration of antibiotics and vitamins in hatcheries as well as by inoculation with contaminated biologics[3]. The introduction of the virus into chicken flocks via commercial vaccines contaminated with this virus has been documented[4,5]. The REV was probably transmitted to the commercial chickens through congenital transmission[6].

The REV infections can cause various pathologic lesions[1]. The most common clinical diseases induced by REV are including runting-stunting disease syndrome, characterized by growth retardation and poor feathering with acute reticular cell neoplasia and chronic B-cell and T-cell lymphomas. The losses and mortalities in affected flocks with REV can be significant due to tumor and or immunosuppression[7]. Although REV is wide spread, REV-induced clinical disease is infrequently diagnosed in commercial flocks[8]. Reticuloendotheliosis cases have been rarely diagnosed in commercial chicken flocks, even though REV infection has been identified in a high proportion of chicken farms by serology and virus isolation[8]. Serological studies have however, indicated that REV infection may be common in commercial chicken flocks[9], although isolation of REV from seropositive chicken flocks has been either unsuccessful or difficult[2,9]. An enzyme-linked immunosorbent assay (ELISA) was successful tool for the detection of avian reticuloendotheliosis[10]. The ELISA has advantages over other tests for the routine detection of avian leukosis virus infection in chickens[11,12]. A microplate indirect enzyme-linked immunosorbent assay (ELISA) for antibodies against reticuloendotheliosis virus (REV) was consistently more sensitive than indirect immunofluorescent-antibody tests and suitable for screening chicken flocks[12].

There are no commercial vaccines are available for control of REV infection and no method has been routinely used by industry to control REV infection in commercial turkey and chicken flocks[13-15]. This study characterized an outbreak of a visceral lymphoma in a commercial cross-bred chicken flock in delta region of Egypt.

MATERIALS AND METHODS

Flock history: Between 2011 and 2012, in 14 cross-bred chicken flocks aged between 12-25 weeks, showed runting, paleness of the face, swelling in the head region, lameness and abnormal feathering as well as tumor-like lesions in the skin, liver and spleen were observed and REV infection was suspected. The farms are located in two provinces in delta Egypt, Kafrelsheikh and Elgharbia provinces. The birds were reared on floor. The birds were vaccinated against Marek's disease, newcastle disease, infectious bronchitis, infectious bursal disease, fowl pox, infectious laryngeotracheitis and avian influenza. Apparent healthy, diseased and dead birds showed above mentioned lesions were submitted to the Laboratory of Poultry Diseases, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt for final diagnosis. Age and number of submitted birds are shown in (Table 1).

Pathological and histopathological examination: Postmortem examination was performed on diseased and freshly dead birds collected from all investigated flocks. In total of 102 suspected tissues as livers, spleens, gizzard and proventriculus were examined for gross and microscopic lesions. The tissue specimens were fixed in 10% neutral buffered formalin solution, embedded in paraffin, sectioned at approximately 5-6 micrometers and stained with Hematoxylin and Eosin (H and E) for microscopical examination[16].

REV antibody detection: In total 12-22 serum samples were collected from each flock for detection of antibodies against REV. Serologic testing was performed by IDEXX REV Ab test using a commercial ELISA kit (IDEXX, Westbrook, ME, USA), according to the manufacturer's instructions. In a flock, if the flock seropositive prevalence lay above 10% was considered to be infected with REV[12].

Hematological studies: Based on the results of serological examination 20 heparinized blood samples from each seropositive and seronegative birds were submitted to hematological examinations.

RESULTS

Clinical and necropsy findings: Affected chickens showed runting, paleness of the face, swelling in the head region and abnormal feathering. Out of submitted 691 birds 278 birds showed tumors (Table 1). The percent of dead birds showing tumor lesions ranged 6.7-51 and 50%. Percentage of birds

Table 1: Investigated farms in this study, total number in each farm, number and age of investigated bird and percentage of birds with tumors

				Birds showing tumor*	
Farm No.	Total No. of birds/flock	Age/week	No. of birds submitted for examination	Number	Percentage (%)
1	2300	12	15	1	06.7
2	2500	13	16	1	06.2
3	3200	14	23	4	17.3
4	2000	15	24	5	20.8
5	4000	16	29	10	34.4
6	5000	17	37	11	29.7
7	2500	18	42	12	28.6
8	2300	19	56	22	29.28
9	4000	20	73	32	43.8
10	5000	21	70	34	48.6
11	2000	22	85	38	44.7
12	2600	23	71	36	50.7
13	2800	24	84	38	45.2
14	2200	25	66	34	51.5
Total			691	278	40.2

Fig. 1: Unusual tumor formation in the head region gross lesions of head region of cross-bred chicken 25 weeks of age with tumors formation

with tumor lesions started at 12th weeks of age and gradually increased in other flocks to reach 28.6% at 18th weeks of age, 44.7% at 22nd weeks of age and 51.5% at 25th weeks of age (Table 1).

The postmortem examination of dead and sacrificed birds revealed moderately to markedly emaciated carcasses, unusual tumor formation in the head region (Fig. 1), congested and enlarged liver, spleen, proventriculus and gizzard with whitish nodular infiltrations in livers and spleens were commonly observed in submitted chickens. In addition, neoplastic nodules in the intestine, pancreas and mesentery could be detected (Fig. 2).

Histopathological pictures: Multiple nodules were observed in the liver, spleen and intestine consisted of homogeneous immature lymphocytes. The head region showed presence of collagen fibers in circular and in parallel or interlake arrangement, hypercellularity, clusters of lipoblasts of varying size, intercellular transudate, dilated blood vessels and aggregation of inflammatory cells surround the blood vessel (Fig. 3a-h).

Some areas were more cellular than others, these cells mainly fibroblasts, which separated from each other by intercellular transudate and focal localized aggregation of inflammatory cells mainly lympho-reticular cells, lymphocytes and histocytes. Aggregation of inflammatory cells surrounded congested blood vessels (Fig. 3). The dilated blood vessels sometimes contain transudate and inflammatory cells (Fig. 3). Mitotic figures of fibroblast were observed (Fig. 4). Clusters of lipoblasts

Fig. 2: Congested and enlarged liver, proventriculus and gizzered with whitish nodular infiltrations organ obtained from chicken with 25 weeks age

Table 2: Reticuloendotheliosis antibody positive percentage in 14 chicken flocks with age ranged from 12-25 weeks using ELISA

Flock No.	Age/week	Positive No./ total examined No.	Percentage (%)
1	12	5/20	25
2	13	12/19	63
3	14	13/20	65
4	15	10/12	83
5	16	17/19	95
6	17	22/23	96
7	18	17/20	85
8	19	23/25	92
9	20	18/19	95
10	21	19/20	95
11	22	14/17	82
12	23	16/16	100
13	24	19/19	100
14	25	22/22	100

of varying size were distributed throughout the tumor mass (Fig. 4). Several nests of tumor giant cell insinuated throughout the tumor mass and hemorrhages were detected (Fig. 5).

Serological investigation: All 14 serologically tested flocks had antibodies to REV. The seropositive prevalence of the REV antibody in cross-bred chicken flocks were 25% (5 out of 20) at 12 weeks of age flock, 65% (13 out of 20) at 14 weeks of age, 83% (10 out of 12) at 15 weeks, 95% (19 out of 20) at 21 weeks, 82% (14 out of 17) at 22 weeks and chicken flock at 25 weeks of age represented significant high ELISA titers against REV 100% (22 out of 22) (Table 2).

Hematological results: The results of blood picture and differential leucocytic count of investigated birds are summarized in Table 3. The total WBCs count revealed significant higher in birds with tumor compared to the birds without lesions. In addition, there were significant differences in heterophils and monocytes count in examined chicken flock as compared with control normal birds.

DISCUSSION

Reticuloendotheliosisis considered as one of the most prevalent pathogens that cause neoplasms in various avian species[1]. The REV has been frequently detected in chickens by serology and virology surveys, few RE field cases have been reported[2,8,9]. Several reports have described RE outbreaks related to vaccination with contaminated FPV[17,18].

The present study demonstrated that REV infection in the field is quite common in poultry flocks in Egypt. Affected chickens suffered from illness, sudden death, abnormal swelling in the head region, runting, mild proventriculitis and

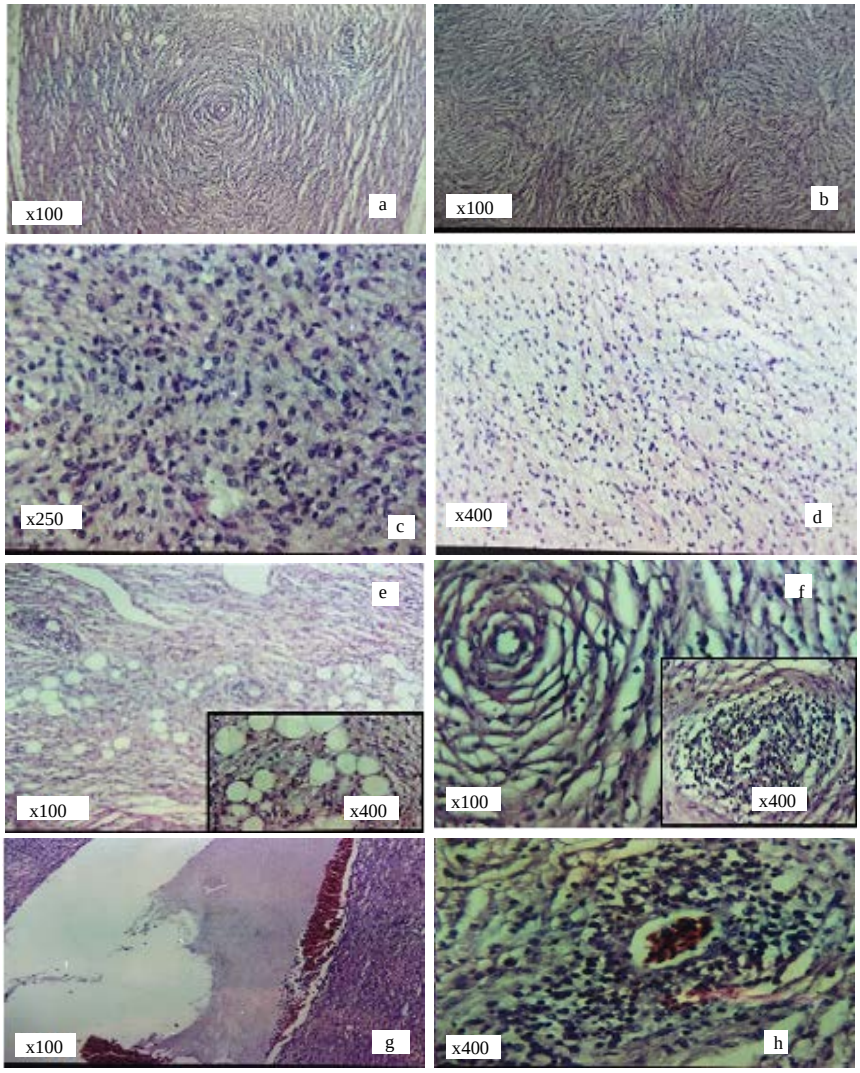

Fig. 3(a-h): Pathological changes in the head showing the (a) Collagen fibers in circular, (b) In parallel or interlake in tumor mass arrangement in tumor mass, (c-d) Hypercellularity in tumor mass, (e) Clusters of lipoblasts of varying size in tumor mass, (f) Intercellular transudate in tumor mass, (g) Dilated blood vessels and (h) Aggregation of inflammatory cells surround the blood vessel

Table 3: Hematological changes in cross-bred chickens during REV outbreak

Parameter	Examined 20 pooled blood samples	Control** 20 pooled blood samples
RBCs count (10^6 mL^{-1}) Mean±SD	2.300±0.0660	2.60±0.350
Hb. conc. (g%) Mean±SD	9.730±0.00	9.24±0.150
Total WBCs count (103 mL^{-1}) Mean±SD	48.630±0.690*	26.75±0.652
Differential leucocytic count (%) Mean±SD		
Heterophils (band)	1.690±0.190	0.00
Heterophils (mature)	28.140±0.780	21.76±1.150
Lymphocytes	65.380±4.890	63.43±1.080
Monocytes	2.740±0.30*	11.34±0.270
Eosinophils	2.600±0.08	2.30±0.270
Basophils	0.700±0.04	1.00±0.014

*Significant different from control at p<0.001 and **Control is cross-bred chicken was serologically negative for REV and clinically healthy birds

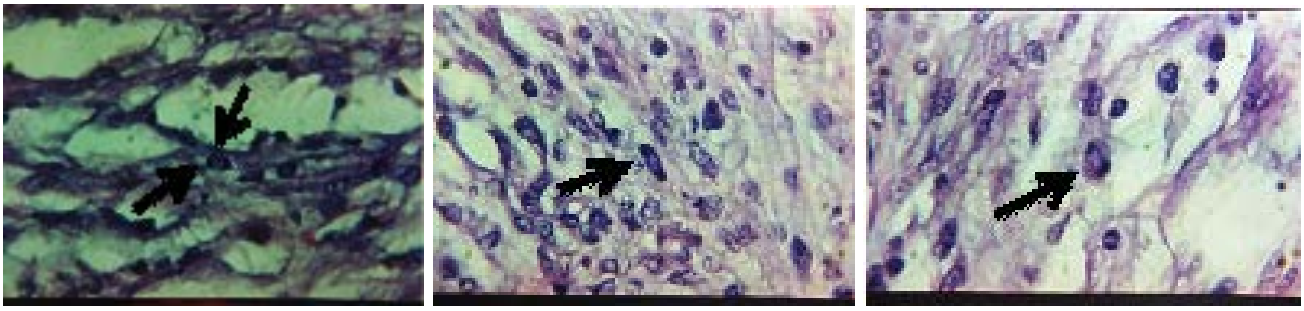

Fig. 4: Section from head region with tumor formation showing mitotic figure of tumor mass (H and E x1000)

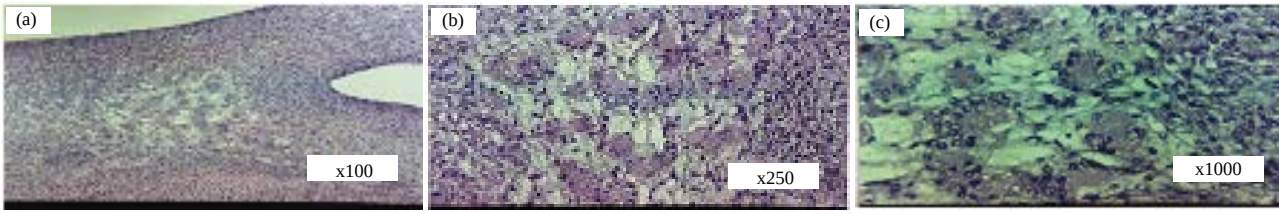

Fig. 5(a-c): Section from head region showing nest of tumor giant cells in tumor mass H and E, (a) x100, (b) x250 and (c) x1000

feathering abnormality. Similar findings were also observed in breeder chicken flocks infected with REV at 13th, 39th and 63rd weeks of age[19]. Mortality due to tumor lesions were demonstrated in sporadic cases at 12th weeks of age and gradually increased in other flocks with advanced age. Percentage of examined birds with tumor reach 28.6% at 18th weeks of age, 42.7% at 22nd weeks of age and 51.5% at 25th weeks of age. These findings are in accordance with previous study conducted in broiler breeder showed bone and visceral tumor[20].

The gross lesions found on autopsy in commercial cross-bred chickens at 12-25 weeks of age, which were creamy and friable tumor in the head, congested and enlarged liver, spleen, proventriculus and gizzard with nodular tumor infiltration. These findings in agreement with previous studies[20,21] conducted in commercial broiler breeder flock, the breeder suffered from visceral and bone tumor due to infection with avian leukosis virus associated with REV and Marek's disease virus infections.

Histopathological examination results revealed that the tumor in the head region consists of solid mass of collagen fibers, fibroblasts and aggregation of inflammatory cells mainly large mononuclear lymphoreticular cells, lymphocytes and histocytes. Mitotic figures of fibroblasts with lipoblasts of varying size with several nests of tumor giant cells insinuated throughout the tumor mass and hemorrhages were detected Similar findings were reported in previous studies[8,21]. Macroscopically, neoplastic nodules were observed in the livers, spleens and intestines of investigated chickens and tissue samples. These findings were similar to those described previously in chickens infected with REV[4,16,22,23]. Lymphoma outbreak of REV infection is closely related to virus dosage and host age[4,24,25]. Based on these results, the pathognomonic lesions were related to REV infection. Finally, a lymphoma outbreak seemed to be caused by REV infection.

The ELISA is more effective at evaluating the presence of REV antibodies than other serologic tests, including agar gel precipitation test and indirect immunofluorescent antibody test[12]. Concerning the serological results, ELISA titers against REV were detected in positive result with a low titer obtained in 12 weeks old positive chicken flock (25%) and then significantly increased to the highest value in a positive flock (100%) at the age 25th weeks. Similar results were demonstrated in study conducted in Taiwan for serological and virological surveys of reticuloendotheliosis in chickens[19]. Moreover, study in USA showed that all tested 6 broiler breeder flocks were infected with reticuloendotheliosis[2]. In contrast to the higher frequency in this study, previous study showed that 14 of 44 broiler breeder flocks in USA were seropositive on a single sampling[9]. Moreover, REV seropositive rates can increase when chickens are infected with a high dose of REV[18,26,27].

Hematological examination revealed significant increase in the total WBCs count and heterophils. Similar results were described in chicken infected with reticuloendothrliosis virus and in broiler breeder chicken infected with avian leucosis virus[2,20], but different from this results in that there was

significant decrease in the number of monocytes in REV-infected chickens as compared with normal control chickens. The significant increase in total WBCs count and the significant difference in heterophils and monocytic count may refer to multi-causal agents of tumor formation.

The findings of this study indicate the wide spread REV infection in commercial cross-bred chickens in Egypt. The serological results suggested circulation of REV within chicken in delta Egypt. Further study is paramount for improving the control measures for REV in chickens.

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REFERENCES

1. Payne, L.N., 1998. Retrovirus-induced disease in poultry. Poult. Sci., 77: 1204-1212.
2. Witter, R.L. and D. Johnson, 1985. Epidemiology of reticuloendotheliosis virus in broiler breeder flocks. Avian Dis., 29: 1140-1154.
3. Fadly, A., 1999. Reticuloendotheliosis Virus Infection in Turkeys: A Review. Proceedings of the 2nd International Symposium on Turkey Diseases, March 24-27, 1999 Berlin, Germany, pp: 47-51.
4. Fadly, A.M., R.L. Witter, E.J. Smith, R.F. Silva, W.M. Reed, F.J. Hoerr and M.R. Putnam, 1996. An outbreak of lymphomas in commercial broiler breeder chickens vaccinated with a fowlpox vaccine contaminated with reticuloendotheliosis virus. Avian Pathol., 25: 35-47.
5. Wozniakowski, G., A. Mamczur and E. Samorek-Salamonowicz, 2015. Common occurrence of *Gallid herpesvirus*-2 with reticuloendotheliosis virus in chickens caused by possible contamination of vaccine stocks. J. Applied Microbiol., 118: 803-808.
6. Wei, K., Z. Sun, S. Zhu, W. Guo and P. Sheng *et al*., 2012. Probable congenital transmission of reticuloendotheliosis virus caused by vaccination with contaminated vaccines. Plos One, Vol. 7. 10.1371/journal.pone.0043422
7. Crespo, R., P.R. Woolcock, A.M. Fadly, C. Hall and H.L. Shivaprasad, 2002. Characterization of T-cell lymphomas associated with an outbreak of reticuloendotheliosis in Turkeys. Avian Pathol., 31: 355-361.
8. Witter, R.L. and A.M. Fadly, 2003. Reticuloendotheliosis, Diseases of Poultry. 11th Edn., Lowa State University Press, USA., pp: 517-536.
9. Witter, R.L., I.L. Peterson, E.J. Smith and D.C. Johnson, 1982. Serologic evidence in commercial chicken and Turkey flocks of infection with reticuloendotheliosis virus. Avian Dis., 26: 753-762.
10. Hafez, H.M., 2001. Serological investigations on reticuloendotheliosis in Turkey flocks. J. Vet. Med. B. Infect. Dis. Vet. Public Health, 48: 547-550.
11. Ignjatovic, J., K.J. Fahey and T. Bagust, 1987. An enzyme linked immunosorbent assay for detection of reticuloendotheliosis virus infection in chickens. Avian Pathol., 16: 609-621.
12. Smith, E.J. and R.L. Witter, 1983. Detection of antibodies against reticuloendotheliosis viruses by an enzyme-linked immunosorbent assay. Avian Dis., 27: 225-234.
13. Davidson, I. and R. Borenshtain, 2001. *In vivo* events of retroviral long terminal repeat integration into Marek's disease virus in commercial poultry: Detection of chimeric molecules as a marker. Avian, Dis., 45: 102-121.
14. Garcia, M., N. Narang, W.M. Reed and A.M. Fadly, 2003. Molecular characterization of reticuloendotheliosis virus insertions in the genome of field and vaccine strains of fowl poxvirus. Avian Dis., 47: 343-354.
15. Hertig, C., B.E.H. Coupar, A.R. Gould and D.B. Boyle, 1997. Field and vaccine strains of fowlpox virus carry integrated sequences from the avian retrovirus, reticuloendotheliosis virus. Virology, 235: 367-376.
16. Koo, B.S., H.R. Lee, E.O. Jeon, H.S. Jang and M.S. Han *et al*., 2013. An outbreak of lymphomas in a layer chicken flock previously infected with fowlpox virus containing integrated reticuloendotheliosis virus. Avian Dis., 57: 812-817.
17. Diallo, I.S., M.A. Mackenzie, P.B. Spradbrow and W.F. Robinson, 1998. Field isolates of fowlpox virus contaminated with reticuloendotheliosis virus. Avian Pathol., 27: 60-66.
18. Fadly, A.M. and R.L. Witter, 1997. Comparative evaluation of *in vitro* and *in vivo* assays for the detection of reticuloendotheliosis virus as a contaminant in a live virus vaccine of poultry. Avian Dis., 41: 695-701.
19. Cheng, W.H., Y.P. Huang and C.H. Wang, 2006. Serological and virological surveys of reticuloendotheliosis in chickens in Taiwan. J. Vet. Med. Sci., 68: 1315-1320.
20. Awad, A., H. Ellakany, A. Eid and H. Abd El-Hamid, 2004. Avian Leukosis Virus subgroup-d (ALV-D) infection in broiler breeder chickens. Proceedings of the 6th Scientific Conference of the Egyptian Veterinary Poultry Association, Volume 9, April 18-22, 2004, Cairo, Egypt, pp: 25-27.
21. El-Gohary, A., H. Sultan, A. El-Sawy and A. Ahmed, 2000. Some epidemiologyical, Haematological and histopatho-logical studies on tumor virus infections in broiler breeders and commercial brown layer chickens. Giza Vet. Med. J., 4: 539-555.
22. Diallo, I.S., J. Taylor, J. Gibson, J. Hoad and A. De Jong *et al*., 2010. Diagnosis of a naturally occurring dual infection of layer chickens with fowlpox virus and gallid herpesvirus 1 (infectious laryngotracheitis virus). Avian Pathol., 39: 25-30.

23. Okoye, J.O.A., W. Ezema and J.N. Agoha, 1993. Naturally occurring clinical reticuloendotheliosis in turkeys and chickens. Avian Pathol., 22: 237-244.
24. Koyama, H., T. Sasaki, Y. Ohwada and Y. Saito, 1980. The relationship between feathering abnormalities. Avian Pathol., 9: 331-340.
25. Wang, J., J. Meers, P.B. Spradbrow and W.F. Robinson, 2006. Evaluation of immune effects of fowlpox vaccine strains and field isolates. Vet. Microbiol., 116: 106-119.
26. Hauck, R., C. Prusas, H.M. Hafez and D. Luschow, 2009. Serologic response against fowl poxvirus and reticuloendotheliosis virus after experimental and natural infections of chickens with fowl poxvirus. Avian Dis., 53: 205-210.
27. Singh, P., T.J. Kim and D.N. Tripathy, 2000. Re-emerging fowlpox: Evaluation of isolates from vaccinated flocks. Avian Pathol., 29: 449-455.