ISSN 1068-3674, Russian Agricultural Sciences, 2018, Vol. 44, No. 5, pp. 477–484. © Allerton Press, Inc., 2018.

VETERINARY

Avian Leukosis in Brahma Chicken Raised in Al-Qurnah Town, Southern Iraq[1]

Qayssar Ali Kraidi[a], *, Waleed Majeed Seger[b], and Mukhallad Abdul Kareem Ramadhan[c]

[a] *Department of Animal Production, Faculty of Agriculture, University of Misan, Misan, Iraq*

[b] *Department of Pathology and Poultry Diseases, Faculty of Veterinary Medicine, University of Basra, Basra, Iraq*

[c] *Department of pathology, Faculty of Medicine, University of Misan, Misan, Iraq*

* *e-mail: qayssarkraidi@yahoo.com*

Received June 13, 2018

Abstract—The current study was conducted to diagnose avian leukosis in naturally infected Brahma backyard chickens in southern parts of Iraq, on the basis of clincopathological findings and serological detection by using antigen-capture enzyme-linked immunosorbent assay (AC-ELISA) in suspected tumor cases in field conditions. In this study the avian leukosis was mostly observed in birds from 16 to 22 weeks of age, as well as the infected flocks showed a low mortality rate ranging from 5–6%. Typical variable sized grey to yellow obvious tumor-like nodular lesion was demonstrated on the surface of enlarged visceral organs such as liver, spleen, kidney and duodenum, as in white meat-type chickens. The histopathological features revealed massive infiltration of monomorphic lymphocytes in which the lymphoblasts were predominant in the liver, kidney, spleen and duodenum. In this study, a total of 40 sera were tested for ALV P27 antigen by ELISA technique. Thirty-five out of forty sera (87.5%) obtained from Brahma chickens tested positive to ALV P27 antigen and a higher percentage (88.58%) of the chicken sera were strongly positive and had (EUs > 75%). Based on these findings, avian leukosis was concluded to be associated with this pathological condition in Iraqi backyard flocks. This is the first report of the presence of the avian leukosis in visceral samples of Brahma breed. It seems that commercial poultry population in Iraq is not far from the threat of the avian leukosis, and surveillance for avian leukosis is needed.

Keywords: avian leukosis, Brahma, chickens, Iraq

DOI: 10.3103/S1068367418050154

INTRODUCTION

Avian leukosis viruses (ALV) are a member of the α-retrovirus genus of retroviridae. Based on its infectivity to different species of birds, interference between different ALV strains in cell cultures, and different antigenicity in cross viral neutralization, ALV are classified into 10 subgroups and based on the envelope glycoprotein (gp85). Exogenous ALV could be classified into different subgroups, namely, A, B, C, D, E, and J. Particularly, the envelope glycoprotein of ALV is responsible for attachment and receptor specificity in addition to the production of neutralizing antibodies [1, 2]. Among them, only A, B, C, D, E, and J subgroups can infect chickens and so far identified, subgroups A, B, and J are considered more prevalent and more economically important [3].

ALV is an avian pathogen that causes neoplastic disease and has also been shown to induce immunosuppression [4] and economically ALV is affecting meat and egg-type breed, subgroup J was first detected in meat-type breed in the UK in 1989 but currently is causing destruction to the poultry industry worldwide [5], there were also some reports of subgroup J myelocytomatosis cases in commercial layer flocks and "yellow" chicken flocks of local breeds in northern China in recent years [6, 7].

The epidemiological, pathological and molecular studies indicate that subgroup J ALV infections are widely spread in some countries [8, 9]. In a histopathological survey, it was reported that lymphoid leukosis was mostly observed in layer birds over 13 weeks of age and not in any of the tissues from broiler birds [10]. Gross examination of tumors was found to be an aid in diagnosing the type of tumors in poultry and histopathology is the best and reliable method for diagnosis of the type of tumors [11]. The researcher suggested that the classical differential diagnosis of avian oncogenic viruses is based on virus isolation and histopathological examination of tumor tissues [12].

Tumor cases and mortality have gradually increased recently in Brahma chickens, which is a large breed of chickens developed in the USA from very large birds imported from the Chinese port of Shanghai, the entry of this type of chickens into Iraq

[1] The article is published in the original.

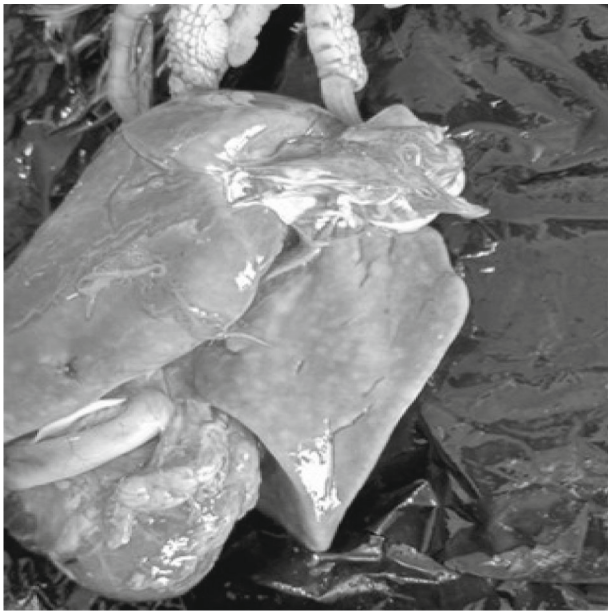

Fig. 1. numerous small, white nodules distributed or the surface of the enlarged liver.

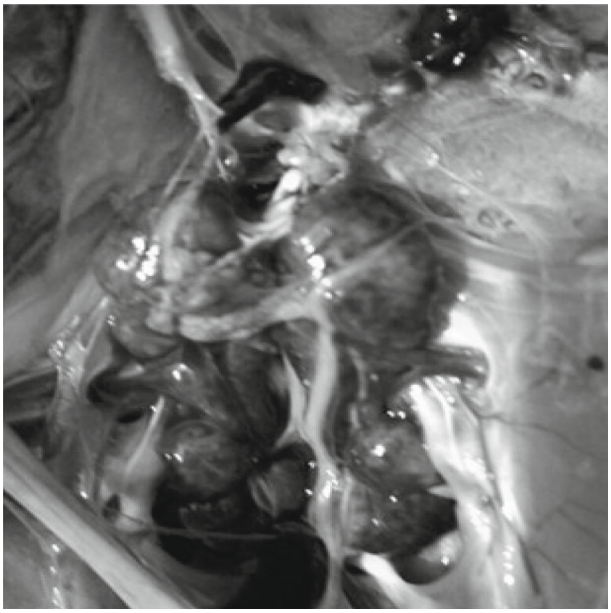

Fig. 2. Kidneys were greatly enlarged and congested with demarcated small whitish nodules.

began by importing it from neighboring countries and become to be raised as a pet and backyard chickens. Therefore, the current study was conducted to diagnose avian leukosis in naturally infected Brahma backyard chickens on the basis of serological and clincopathological findings in suspected tumor cases in field conditions.

MATERIALS AND METHODS

Flock History

All four small backyard flocks investigated in this study were located in the Al-Qurnah (a town in southern Iraq about 74 km northwest of Basra province) with Brahma chickens breeds raised in open houses. In these flocks, the mortality and tumor cases have gradually increased during the period from November 2017 to January 2018. The birds of these flocks with various ages were presented to the department of veterinary Pathology and poultry diseases at the University of Basra for necropsy examination. The information such as the detailed history of clinical signs, mortality and age of chickens were recorded. These flocks had no history of vaccination against ALV. All the suspected birds were necropsied systematically as per the standard procedure.

Histopathological Examination

Tissue samples from internal organs including (liver, kidney, spleen and duodenum) were collected and preserved in 10% formalin solution for histopathological examination. The formalin-fixed tissues were processed by routine paraffin embedding and 4 to 5 μm sections were stained with hematoxylin and eosin stain by employing standard procedures.

Blood Samples

A total of 40 blood samples from 4 flocks (10 samples for each) were collected and used for detection of ALV by serological examination.

ELISA

An Antigen-capture enzyme-linked immunosorbent assay (AC-ELISA) (IDEXX Laboratories, Inc., Westbrook, Maine) was used to test all (40) sera according to the manufacturer's instructions. With this kit, optical density (OD) values were transformed to S/P ratios based on the OD for the serum sample together with those for the negative and positive controls provided with the kit by using the following equation: S/P ratio = (OD of sample – OD of negative control)/(OD of positive control – OD of negative control). The ELISA units (EUs) were calculated as S/P ratio ×100. All assays were run in duplicate.

RESULTS

Clinical Signs

In the present study, birds suspected for avian leukosis have been reported in infected flocks from 16 to 22 weeks of age and the clinical signs of some live birds submitted for necropsy had decreased growth rates, depression, inappetence, dehydration and emaciation with low mortality rate ranging from 5–6%.

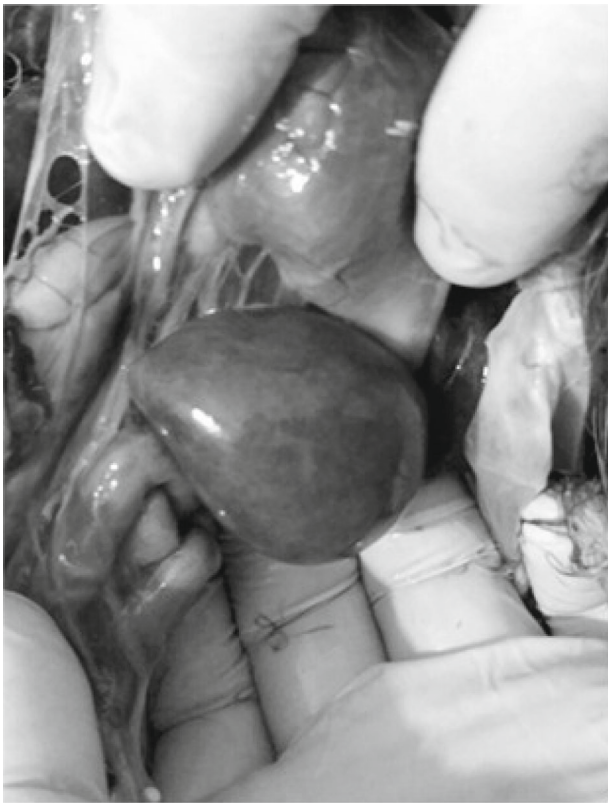

Fig. 3. Severe enlargement of the spleen accompanied by diffuse leukosis.

Fig. 4. Duodenum enlarged, thickened with areas of leukosis.

Gross Lesions

Out of the 20 examined birds suspected for avian leukosis were inspected thoroughly at necropsy for the presence of tumorous lesions in different organs, fifteen (75%) cases demonstrated variable sized grey to yellow obvious tumour-like nodular lesion distributed on the surface of enlarged visceral organs such as liver, kidney, spleen and duodenum (Figs. 1–4). The tumors were soft, smooth and glistening and the cut surface was greyish to creamy white with areas of necrosis. The enlargement and lesions were found to be more pronounced in the liver accompanied by a nodular, miliary and diffuse pattern of tumorous growths. Kidneys were greatly enlarged and congested with demarcated small whitish nodules. Duodenum enlarged, thickened with areas of leukosis. Spleens were grossly enlarged with diffuse leukosis.

Histopathological Lesions

Liver. Histopathological changes in the liver showed focal infiltration of monomorphic lymphocytes in which the lymphoblasts were predominant. These changes involved most of the hepatic lobules, which appears to contain only cords of hepatocytes as the rest of each lobule infiltrated with lymphocytes (Figs. 5, 6).

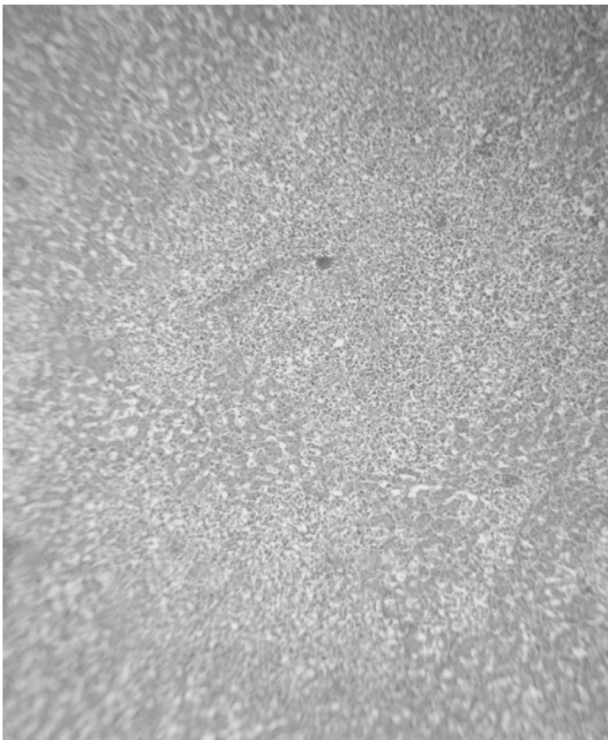

Fig. 5. Section of liver shows focal infiltration of lymphocytes in the hepatic parenchyma. 125× H&E.

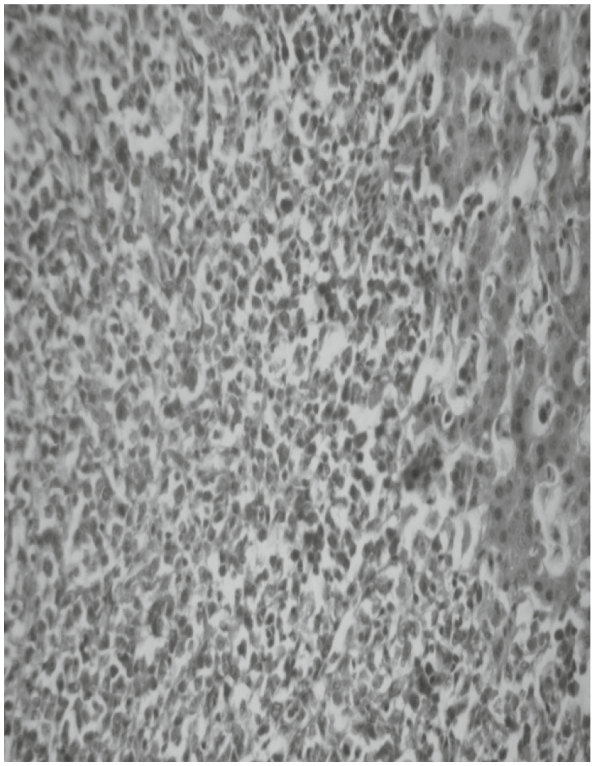

Fig. 6. Section of liver on high magnification power reveal uniformed lymphocytes with lymphoblasts predominance 500× H&E.

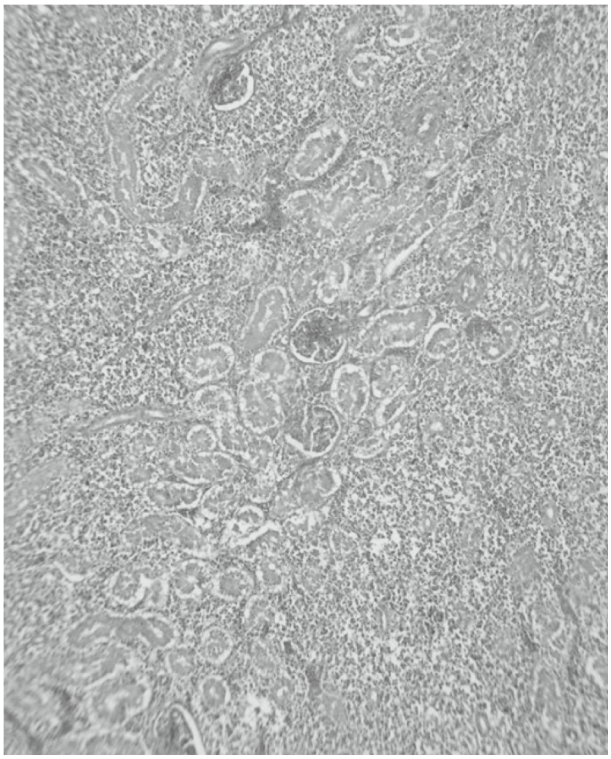

Fig. 7. The kidney shows massive destruction of renal parenchyma with marked invasion of lymphocytes 125× H&E.

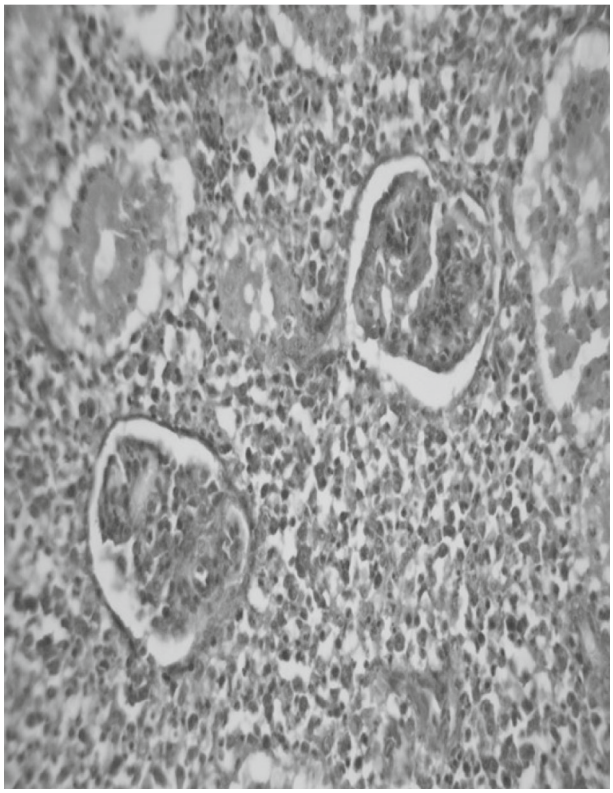

Fig. 8. The kidney reveal massive infiltration of lymphocytes mostly monomorphic lymphoblasts 500× H&E.

Kidney. Kidney section revealed massive interstitial infiltration of lymphocytes and lymphoblast's, with degeneration of renal tubular epithelial cells and proliferation of glomerular mesangial cells (Figs. 7, 8).

Spleen. Heavy infiltration of lymphoid cells consists of mixture of a mature and immature lymphocytes. The white pulp of the spleen appears to be expanded by the lymphoid infiltration with remission of the red pulp (Figs. 9, 10).

Duodenum. The lymphoid cells appear also to invade the intestinal mucosa and sub-mucosa in which the lamina propria of the intestinal villi is flooded with lymphoid cells. Intestinal villi reveal epithelial sloughing (Figs. 11, 12).

ELISA

ELISA units (EUs) less than 10 were considered negative for ALV P27 antigen. EUs 10–25 were considered weakly positive, while EUs 25–75 were considered moderately positive, while EUs greater than 75 were considered as strongly positive for ALV P27 antigen. In this study, a total of 40 sera were tested for ALV P27 antigen by ELISA technique. Thirty-five out of forty sera obtained from Brahma chickens tested positive to ALV P27 antigen which represents 87.5%. Three (8.57%) of the thirty-five sera were weakly positive

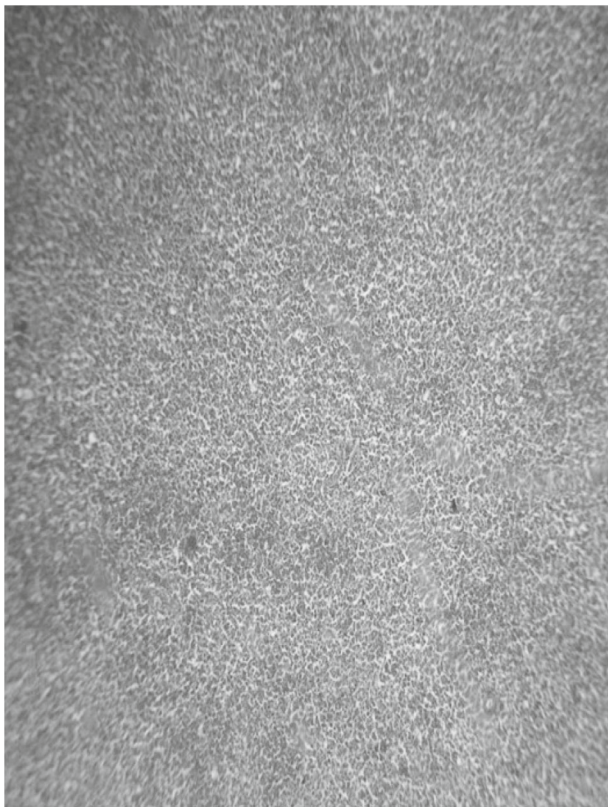

Fig. 9. Section of spleen shows diffuse heavy infiltration of lymphocytes in which it causes marked expansion of the white pulp. 125× H&E.

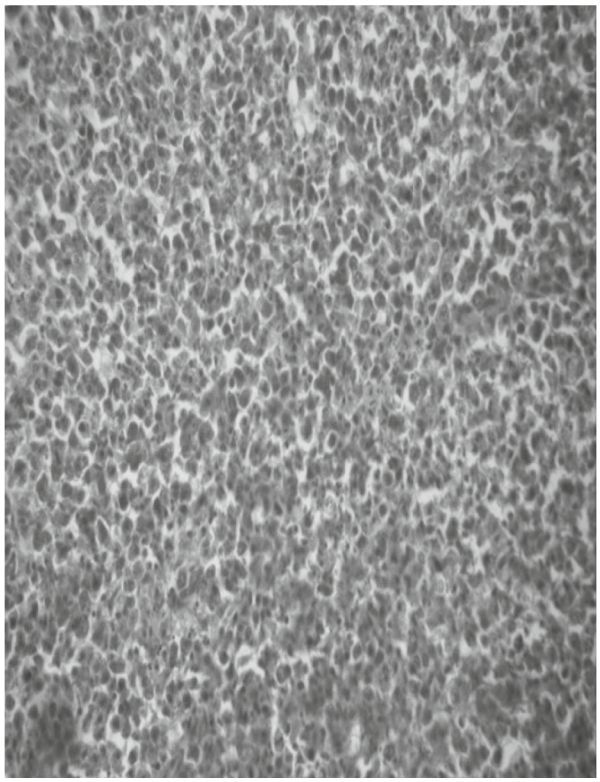

Fig. 10. Section of spleen shows that the lymphocytes are of slightly varying sizes of large and small lymphocytes. 500× H&E.

(EUs < 10%) to ALV P27 antigen, while one (2.85%) serum sample was moderately positive (EUs 25–75%) to ALV P27 antigen, while thirty-one (88.58%) sera were strongly positive (>75%) to ALV P27 antigen (Table 1).

DISCUSSION

The Brahma (Shanghai birds) was the principal meat breed in the US from 1850 to 1930. Birds were very large, weighing about 8 kg for cocks and 6 kg for hens with average weights about 5.5 kg for cocks and 4.5 kg for hens were recorded. Also, the Brahma is a good winter layer of large brown eggs; eggs weigh approximately 55–60 g [13]. These days, people raise Brahma chickens for both egg and meat production and also for the ornamental purpose. For this reasons, it has become a favorite type of chickens in the backyard flocks in Iraq particularly in southern parts of Iraq. During the period of the current study, tumor cases and mortality have gradually increased recently in Brahma chickens in Iraq. ALV, the major oncogenic viral pathogen of Retroviridae family responsible for mortality and huge economic loss to the developed poultry industry even until today. Little is known about the avian leukosis in naturally infected backyard chickens in Iraq.

Regarding the clinical signs, the infected bird had reduced growth rates, depression, inappetence, dehydration and emaciation. These findings are in line with findings of previous studies [14, 15], as well as the infected flocks showed a low mortality rate ranging from 5–6% and these results are similar to the results of [16] who reported 7 to 10% mortality rate in the Chinese local "yellow" chickens infected with subgroup J of ALV. In spite of the fact that quantity and quality of egg production are decreased in the laying hens associated with infection with ALV [17], the decline in egg production or eggs of reduced size and quality were not reported in this study and this result might be attributed to the timing of the diagnosis of avian leukosis which was in pre-period of egg production, as it is known that Brahma hens usually start laying eggs after their six or seven months of age.

Fifteen (75%) suspected cases grossly demonstrated variable sized grey to yellow obvious tumour-like nodular lesion distributed on the surface of enlarged visceral organs, but they were more common in the liver, spleen, kidney and duodenum. These findings are in accordance with the earlier results of [16, 18] who reported 80 and 65.38% tumor-like nod-

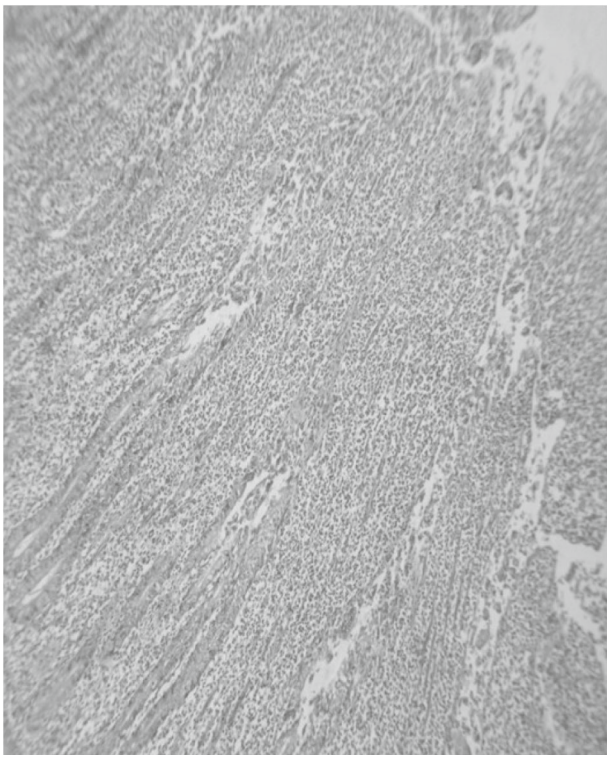

Fig. 11. Section of intestine shows migration of lymphocytes in the mucosa and submucosa with sloughing of the intestinal epithelium. 125× H&E.

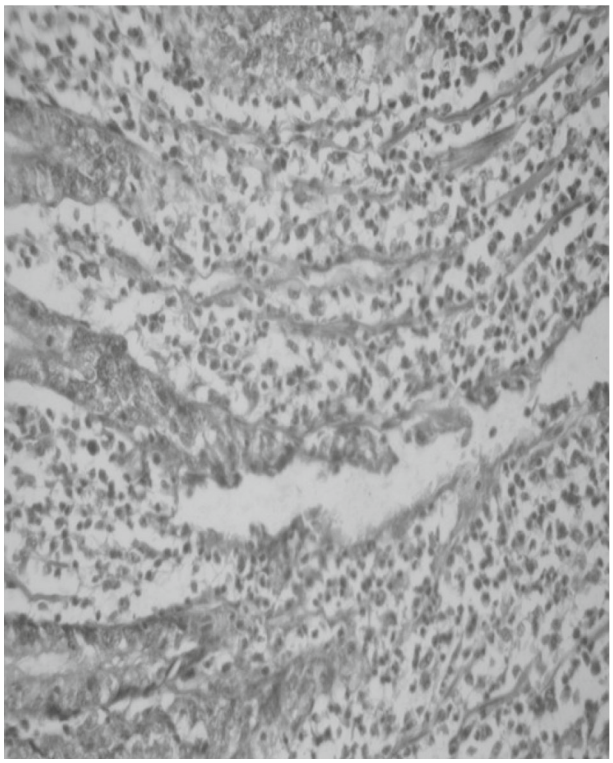

Fig. 12. Section of intestine shows infiltration of lymphocytes in the lamina propria. 500× H&E.

ular lesions in visceral organs of chicken with avian leukosis in China and India, respectively.

All cases were suspected for avian leukosis depending upon gross lesion of internal organs, were confirmed for avian leukosis based upon histopathological features, which have included massive infiltration of uniform sized lymphoid cells and lymphoblasts in the examined sections of internal organs, these features were in coincidence with the earlier observation [18, 19]. It seems that the histopathological examination is still found to be an excellent tool for diagnosis of tumor cases with avian leukosis. In particular, a typical gross and histological lesions detected in eight necropsied Chinese local "yellow" chickens suspected of avian leukosis, and ALV was isolated and identified from seven of these eight birds with tumors [16].

In this study, a total of 40 sera were tested for ALV P27 antigen by ELISA technique. Thirty-five out of forty sera (87.5%) obtained from Brahma chickens tested positive to ALV P27 antigen. Since higher percentage (88.58%) of the chicken sera were strongly positive and had (EUs > 75%) to ALV P27 antigen, it is clear that there was a severe infection in those four Brahma flocks. According to the results of the present study, this is the first report of the presence of the avian leukosis in Brahma chickens which are recently being raised in Iraq as backyard chickens. The origin of avian leukosis in Iraq is not clear but several scenarios were proposed for the introduction of avian leukosis into poultry in Iraq. (i) purchasing of infected birds from neighboring countries and smuggle them to Iraq, (ii) ALV contamination in a live vaccine, such as contaminated live Newcastle Disease virus vaccine with subgroup A ALV in China [20], (ii) undetected long term occurrence of the avian leukosis in Iraq, particularly the commercial egg type bird sell in live chicken markets at the end of the laying cycle and mixing with backyard chickens which may provide a good opportunity for transmission of ALV between different breed of bird, its well documented that ALV can be transmitted horizontally and vertically among the chickens in the infected flocks [21].

CONCLUSIONS

Despite the implementation of several successful eradication programmes and development of breeding lines of chickens resistant to avian leukosis in recent years. However, avian leukosis started to spread in different types of chickens, such as the Taiwan native breed crossbred with imported white meat-type breeders, and old fancy chicken breeds in the Netherlands [22, 23] and in some flocks of Chinese local chicken breeds [7]. Based on these findings, avian leukosis was concluded to be associated with this pathological condition in Iraqi backyard flocks, and affected chickens had reduced growth rates, depression, inappetence,

Table 1. Percentage of positive results to ALV P27 antigen by ELISA S/P and EUs range

Sample No.	Mean OD	S/P	EUs	ALV
1	0.57	0.98	97.77	S+
2	0.52	0.88	87.63	S+
3	0.54	0.92	91.68	S+
4	0.55	0.94	93.71	S+
5	0.51	0.86	85.60	S+
6	0.45	0.73	73.43	S+
7	0.21	0.25	24.75	W+
8	0.53	0.90	89.66	S+
9	0.54	0.92	91.68	S+
10	0.55	0.94	93.71	S+
11	0.51	0.86	85.60	S+
12	0.51	0.86	85.60	S+
13	0.5	0.84	83.57	S+
14	0.12	0.06	6.49	N–
15	0.51	0.86	85.60	S+
16	0.53	0.90	89.66	S+
17	0.55	0.94	93.71	S+
18	0.13	0.09	8.52	N-
19	0.54	0.92	91.68	S+
20	0.1	0.02	2.43	N-
21	0.55	0.94	93.71	S+
22	0.52	0.88	87.63	S+
23	0.5	0.84	83.57	S+
24	0.57	0.98	97.77	S+
25	0.52	0.88	87.63	S+
26	0.11	0.04	4.46	N-
27	0.5	0.84	83.57	S+
28	0.52	0.88	87.63	S+
29	0.51	0.86	85.60	S+
30	0.48	0.80	79.51	S+
31	0.47	0.77	77.48	S+
32	0.2	0.23	22.72	W+
33	0.1	0.02	2.43	N-
34	0.49	0.82	81.54	S+
35	0.48	0.80	79.51	S+
36	0.51	0.86	85.60	S+
37	0.19	0.21	20.69	W+
38	0.33	0.49	49.09	M+
39	0.56	0.96	95.74	S+
40	0.53	0.90	89.66	S+

S+ = strongly positive sample; N– = negative sample; W+ = weakly positive; M+ = moderately positive sample.

dehydration and emaciation. It seems that commercial poultry population in Iraq is not far from the threat of the avian leukosis, and surveillance for avian leukosis is needed. Furthermore, it is essential that the biosecurity on poultry flocks should be improved to prevent the introduction and dissemination of avian leukosis.

ACKNOWLEDGMENTS

The authors would like to acknowledge from the staff of department of Veterinary Pathology and poultry diseases, University of Basra for providing the support and facility for carrying out the research work.

REFERENCES

1. Coffin, J.M., *Structure and Classification of Retroviruses,* Springer, 1992, pp. 19–49.
2. Payne, L., Howes, K., Gillespie, A., and Smith, L., Host range of Rous sarcoma virus pseudotype RSV (HPRS-103) in 12 avian species: Support for a new avian retrovirus envelope subgroup, designated J, *J. Gen. Virol.*, 1992, vol. 73, no. 11, pp. 2995–2997.
3. Dai, M., Feng, M., Liu, D., Cao, W., and Liao, M., Development and application of SYBR Green I real-time PCR assay for the separate detection of subgroup J Avian leukosis virus and multiplex detection of avian leukosis virus subgroups A and B, *Virol. J.,* 2015, vol. 12, no. 1, p. 52.
4. Lütticken, D., Viral diseases of the immune system and strategies to control infectious bursal disease by vaccination, *Acta Vet. Hung.,* 1997, vol. 45, no. 3, pp. 239–249.
5. Payne, L., Brown, S., Bumstead, N., Howes, K., Frazier, J.A., and Thouless, M.E., A novel subgroup of exogenous avian leukosis virus in chickens, *J. Gen. Virol.,* 1991, vol. 72, no. 4, pp. 801–807.
6. Xu, B., Dong, W., Yu, C., He, Z., Lv, Y., Sun, Y., Feng, X., Li, N., Lee, L., and Li, M., Occurrence of avian leukosis virus subgroup J in commercial layer flocks in China, *Avian Pathol.,* 2004, vol. 33, no. 1, pp. 13–17.
7. Cheng, Z., Zhang, L., Liu, S., Zhang, L., and Cui, Z., Emerging of avian leukosis virus subgroup J in a flock of Chinese local breed, *Acta Microbiol. Sin.,* 2005, vol. 45, no. 4, pp. 584–587.
8. Sun, S. and Cui, Z., Epidemiological and pathological studies of subgroup J avian leukosis virus infections in Chinese local "yellow" chickens, *Avian Pathol.,* 2007, vol. 36, no. 3, pp. 221–226.
9. Bande, F., Arshad, S.S., and Omar, A.R., Isolation and metagenomic identification of avian leukosis virus associated with mortality in broiler chicken, *Adv. Virol.,* 2016, vol. 2016, pp. 1–4.
10. Balachandran, C., Pazhanivel, N., Vairamuthu, S., and Murali Manohar, B., Marek's disease and lymphoid leucosis in chicken—a histopathological survey, *Tamilnadu J. Vet. Anim. Sci.,* 2009, vol. 5, no. 4, pp. 167–170.
11. Swathi, B., Kumar, A.A., and Reddy, M., Histological and molecular diagnosis of poultry tumours, *Indian J. Vet. Pathol.,* 2012, vol. 36, no. 1, pp. 41–48.

12. Davidson, I. and Borenstein, R., Multiple infection of chickens and turkeys with avian oncogenic viruses: Prevalence and molecular analysis, *Acta. Virol.,* 1999, vol. 43, nos. 2–3, pp. 136–142.

13. Hrnčár, C., Hässlerová, M., and Bujko, J., The effect of oviposition time on egg quality parameters in Brown Leghorn, Oravka and Brahma hens, *Sci. Pap. Anim. Sci. Biotechnol.,* 2013, vol. 46, no. 1, pp. 53–57.

14. Burmester, B., Studies on the transmission of avian visceral lymphomatosis. II. Propagation of lymphomatosis with cellular and cell-free preparations, *Cancer Res.,* vol. 7, no. 12, pp. 786–797.

15. Abdel-Latif, M.M. and Khalafalla, A.I., Detection by PCR of multiple subgroups of avian leukosis virus (ALV) in broilers in the Sudan, *J. Anim. Vet. Adv.,* 2005, vol. 4, no. 3, pp. 407–413.

16. Sun, S. and Cui, Z., Epidemiological and pathological studies of subgroup J avian leukosis virus infections in Chinese local "yellow" chickens, *Avian Pathol.,* 2007, vol. 36, pp. 221–226.

17. Gavora, J., Spencer, J., Gowe, R., and Harris, D., Lymphoid leukosis virus infection: Effects on production and mortality and consequences in selection for high egg production, *Poult. Sci.,* 1980, vol. 59, no. 10, pp. 2165–2178.

18. Sagarika, S., Das, D., Panda, S.K., Das, S., and Sahu, B.J.R.K., Avian leukosis in chickens: A clinicopathological survey, *J. Entomol. Zool. Stud*., 2017, vol. 5.

19. Gopal, S., Manoharan, P., Kathaperumal, K., Chidambaram, B., and Divya, K.C., Differential detection of avian oncogenic viruses in poultry layer farms and turkeys by use of multiplex PCR, *J. Clin. Microbiol.,* 2012, vol. 50, no. 8, pp. 2668–2673.

20. Zhao, P., Dong, X., and Cui, Z., Isolation, identification, and gp85 characterization of a subgroup A avian leukosis virus from a contaminated live Newcastle Disease virus vaccine, first report in China, *Poult. Sci.,* 2014, vol. 93 no. 9, pp. 2168–2174.

21. Li, Y., Cui, S., Li, W., Wang, Y., Cui, Z., Zhao, P., and Chang, S., Vertical transmission of avian leukosis virus subgroup J (ALV-J) from hens infected through artificial insemination with ALV-J infected semen, *BMC Vet. Res.,* 2017, vol. 13, no. 1, p. 204.

22. Thu, W.-L. and Wang, C.-H., Phylogenetic analysis of subgroup J avian leucosis virus from broiler and native chickens in Taiwan during 2000–2002, *J. Vet. Med. Sci*., 2003, vol. 65, no. 3, pp. 325–328.

23. Crooijmans, R. and Pijpers, A., A serological survey for pathogens in old fancy chicken breeds in central and eastern part of the Netherlands, *Tijdschr. Diergeneeskd.,* 2004, vol. 129, no. 10, pp. 324–327.