Vet.Med.J.,Giza. Vol.54, No.3. (2006): 563-577.

PATHOLOGICAL AND MOLECULAR STUDIES ON AVIAN LEUKOSIS VIRUS SUBGROUP (J) IN CHICKEN PARENTS FLOCKS

OSMAN, A. H.*; EL-MAHDY, M. M.*; MONA, M. A** and SOLIMAN M.A**.
*Department of Pathology, Faculty of Veterinary Medicine, Cairo University.
**Animal Health Research Institute-Dokki.

Received: 15. 3. 2006.
Accepted: 3. 4. 2006.

SUMMARY

The present study was carried out on 10 parent chickens flocks collected from different provinces. The examined cases were classified into 47 commercial layers and 198 broiler parents to study the gross and microscopical lesions of different tumors induced by avian leukosis subgroup-J (ALV-J) in broiler breeders and layers flocks.

Serological tests were conducted on 245 serum samples to detect the antibodies of ALV-J. Polymerase chain reaction PCR was carried out on tissue samples to confirm the presence of DNA of ALV subgroup J. Myeloid leukosis was recorded in four broiler flocks induced by the novel subgroup-J of ALV.

Pathological findings of infected parent chickens flocks revealed multiple neoplastic nodules in different organs including liver, kidney, gonads, intestine, skeletal muscle and bone. Histologically, the main constituent of tumor lesions was mature and immature myelocytes. Nephroblastoma was considered as the first recorded cases in Egyptian broiler parent flocks affected with ALV subgroup J.

The results of this work concluded that the histopathological examination plays a crucial and decisive role in diagnosing different types of neoplasm caused by avian leukosis virus subgroup-J. The application of PCR test verified the presence of DNA of ALV-J that induced the tumors of myeloid leukosis.

INTRODUCTION

Leukosis/sarcoma (L/S) group designated a variety of transmissible benign and malignant tumours of chickens caused by members that belong to the family Retroviridae Regenmortel et al. (2000). The broad host range pattern of (HPRS-103 strain) differs from those of viruses of subgroups A to G and I and provided a support for placing the strain HPRS-103 of ALV-J in a new envelope subgroup, designated as subgroup (J) Payne et al. (1992).

Enzyme linked immunosorbent assay (ELISA) was used for the detection of antibody to the gp85 envelope protein of ALV-J. The test was of value for large-scale screening programs for the presence of ALV-J infection Fuchs et al. (2000). The PCR test was considered as accurate tool for detection of subgroup- J ALV. This test depended on that the env gene sequence of ALV subgroup J was distinct from other subgroups by using a down steam primer derived from HPRS-103 env sequence. Smith et al. (1998).

Histopathological investigations revealed typical lesions of myelocytomatosis. Gross lesions of myeloid leukosis tumors were seen in several organs included liver, spleen, thymus, gonads and kidneys. The tumor masses were also observed in skeletal muscle, inner sternum, ribs, vertebrae and synsacrum Payne (1998). The morphology of the tumor cells can be verified in sections or smears stained with Romanowsky or May- Grünwald-Giemsa stains. The tumors consisted of uniformly differentiated mature myelocytes, whose cytoplasm was filled with acidophilic round granules Nakamura et al. (2000); Mona, (2000) and El-Gohary et al. (2000).

MATERIAL AND METHODS

The present study was conducted on 245 commercial layers and broiler parents received from different governorates for diagnosis of avian tumors during 2004- 2005 as shown in table (1).

Table (1) showing the number of examined birds and their geographical distribution.

Flock no.	Governorate	Type of flock	Age of bird	No. of birds
1	Giza	Broiler parent	32 weeks	23
2	Ismailia	Broiler parent	38 weeks	25
3	Behera	Broiler parent	28 weeks	20
4	Behera	Broiler parent	26 weeks	20
5	Kaliobia	Layers	36 weeks	22
6	Giza	Layers	35 weeks	25
7	Behera	Broiler parent	35 weeks	25
8	Behera	Broiler parent	30 weeks	30
9	Behera	Broiler parent	40 weeks	30
10	Dakahlia	Broiler parent	32 weeks	25

Sampling:

Blood Samples:

A total of 245 blood samples were collected from 2 commercial layers and 8 broiler parents flocks. Blood samples were left for agglutination overnight and then centrifuged at 3000 rpm /10min. to separate the serum for detection of antibodies of ALV and ALV- J by ELISA test as shown in table (2).

ELISA test for detection of ALV& ALV-J antibodies in serum samples:

A total of 245 serum samples were tested for the presence of ALV (A, B) & ALV-J antibodies. The procedure recommended by the manufacturers (IDEXX, labo-ratories, Inc., Maine, and USA) was applied according to (Venugopal et al., 1997)

Table (2): Showing the number of blood samples collected for Serological test:

Type of chickens	No. of flocks	No. of samples
Layers	2	47
Broiler parents	8	198
Total	10	245

Tissue Samples:

Tissue specimens collected from the examined chickens were divided into two parts. The first part of tissue samples were fixed in neutral buffered formalin 10% for histopathological examination. The second part of tissue samples were collected from different organs showed tumor lesions and frozen at - 70° C for polymerase chain reaction PCR test.

Histopathological examination:

Tissues from liver, kidneys, heart, skeletal muscle, ovaries, bursa of Fabricius, spleen, and intestine were taken from examined chickens and fixed in 10% neutral buffered formalin. The fixed specimens were then trimmed, washed, dehydrated in ascending grades of alcohols, cleared in xylene, embedded in paraffin, sectioned at 4-6µ thickness and stained with haematoxylin & eosin. Moreover, special stains were used. Methyl green Pyronin and May - Grünwald - Gimsa Bancroft & Cook (1993).

PCR assay for detection of ALV-J DNA genome in tissues homogenates:

PCR test was conducted on DNA extracted from tissues that showed tumor lesions in 6 flocks which given positive ELISA test for ALV-J as recorded in table (3). The concentration of DNA was determined by measuring of absorbency at 260 nm and template concentration adjusted to 50ug/ml (Murray & Thompson 1980). Reddy Mix PCR - Master Mix PCR reagent Kit with amplifier Taq DNA polymerase. (PCR reagents are manufactured by AB gene laboratories, Surrey, UK) (Lot No. 0311/10). Primers: the specific sequences of nucleotides for ALV-J used in the study forward & reverse were H5/H7 respectively as described by (Smith et al., 1998).

PCR program was consisting of the following steps: The amplification: denaturation at 93°C for 1min., annealing at 60°C for 1min. decreasing by 1°C in each cycle and extension at 72°C for 90 seconds to 13 cycles followed by 30 cycles of 93°C for 1 min., 48°C for 1 min., 72°C for 90 sec. with final extension at 72°C for 10 min. Reactions were conducted in Thermocycler. The amplification products were analyzed by gel electrophoresis was prepared by 1.5 gm ultra pure agarose added to 100ml TBE then melted in hot air oven. Products were stained with ethidium bromide (0.5 ul per ml of gel). The stained amplified products were observed under ultra violet transluminator and photographed by Polaroid Camera (Smith et al. 1998).

Table (3): Showing the number of examined flocks for DNA detection of ALV-J using PCR test:

Type of chickens	No. of examined flocks
Layers	2*
Broiler parents	4**
Total	6

* Flocks no. 1, 2, 4 and 10.
** Flocks no. 6,7.

RESULTS

Pathological findings:-

Liver:

The liver of infected birds was greatly enlarged occupying most of the abdominal cavity. Multiple elevated creamy nodular subcapsular masses were seen (Fig. 1).

The hepatic lobules showed focal aggregations of mature and immature granulated myelocytes adjacent to the blood vessels (Fig. 2). The hepatocytes were replaced by neoplastic cells with atrophy of hepatic cords. The portal triads were infiltrated with large number of proliferating myelocytes. The bile ducts were lined by cuboidal or columnar epithelium and mucous secreting cells (Fig. 3). These myelocytic cells were characterized by eccentrically located nuclei and marked nucleolus with clear mitotic figure. The cytoplasm was filled with conspicuous spherical eosinophilic granules.

Kidneys:

The kidneys of infected birds showed grayish white patches of variable size and shape. Subcapsular haemorrhagic areas were also seen. The kidney appeared friable with marked enlargement of its lobes compared with non infected birds (Fig. 4).

Microscopically, kidneys of infected birds showed focal aggregations of immature and mature granular myelocytes in both cortex and medulla. The tumor cells were aggregated usually around the dilated blood vessels (Fig. 5). The renal tubules showed sloughing of its epithelial lining together with the presence of flocculated

intra-luminal protineous material. Some renal tubules showed cellular cast consisted of desquamated epithelial cells intermingled with myelocytes (Fig.6).

Heart:

The thoracic cavity showed yellowish white, soft and friable nodular or diffuse masses. These tumour masses were seen in costochondral junctions of the ribs, the inner surface of sternum and cardiac muscle (Fig.7).

The cardiac muscle showed nodular lesions consisted of massive aggregations of immature and mature granular myelocytes. Perivascular oedema with myeloid cells infiltration was also seen (Fig.8).

Skeletal Muscles:

The skeletal muscles adhered to the flat bones especially the sternum, ribs and synsacrum revealed grayish white, soft and friable elevated nodules of variable sizes (Fig.9).

Stained section of the pectoral muscle of the infected birds revealed Zenker's necrosis of muscle bundles which appeared as homogenous eosinophilic structureless masses separated by mature and immature myelocytic cells infiltrations (Fig.10).

Bone and Cartilage:

Some infected birds showed typical features of myelocytomatosis characterized by yellowish white nodules on the surface of sternum, ribs and synsacrum.

The cartilaginous part of sternum revealed large numbers of proliferating mature and immature granulated myelocytes in diffuse manner around the degenerated cartilage (Fig.11).

Intestine:

The intestinal mucosa revealed grayish white elevated areas scattered along the intestinal tract. The intestinal lumen was filled with yellowish slimy fluid with offensive odour.

Microscopically, the intestinal mucosa showed sloughing of its epithelial lining. The lamina propria was infiltrated with large number of mature and immature myelocytes. The central lactael were dilated and surrounded by uniformly differentiated mature myelocytes (Fig.12). The submucosal connective tissue showed perivascular oedema and also myelocytic infiltration.

Ovary:

Most of the examined cases were suffering from decreased egg production associated with atrophy of ovarian follicles (Fig.13).

The ovary of infected bird showed focal aggregations of mature and immature myelocytic cells which occupied most of the ovarian parenchyma. The myelocytic cells surround the atrophied ovarian follicles and perivascular spaces (Fig.14).

These myelocytic cells showed a characteristic brilliant red colored cytoplasmic granules when stained with May - Grünwald - Gimsa stain) (Fig. 15).

Miscellaneous tumor

Nephroblastoma:

Nephroblastoma was considered as highly malignant embryonic tumor. This type of tumor was recorded in two examined birds infected with ALV-J. The kidneys of infected birds showed spherical well demarcated tumor mass embedded in its parenchyma. On cut section, the tumour appeared soft, friable, grayish white with hemorrhagic spots.

The tumour mass was separated from apparently healthy tissue by thick fibrous connective tissue and leukocytic infiltration (Fig.16). The neoplastic mass showed undifferentiated cystic renal tubules with intra-luminal protineous cast (Fig. 17). The interstitial tissue showed polymorph stromal cells which appeared round or stellate shape. Structures like glomeruli or metanephr precursor of glomeruli were observed. The ren tubules were lined by undifferentiated cuboidal columnar epithelium and merged with strom cells (Fig. 18).

Results of serological test (ELISA):

ELISA test was applied on 245 serum samples of commercial layers and 198 of broiler parent for detection of ALV-J antibodies. The age wa ranged from 26 up to 50 weeks. ELISA test re vealed positive results in 6 out of 8 broiler paren flocks with an incidence of (19/23) 83%, (20/25 80 %, (14/20) 70%, (15/20) 75%, (21/30) 70% and (18/25) 72% respectively and a total inci dence (107/245) 45.7% among broiler chicken On the other hand commercial layers reacted neg atively with (ELISA) test as shown in table (4).

Table (4): Results of examined sera samples for detection ALV- J antibodies by using ELISA:

No.	Province	Type	Age (weeks)	No. of samples	Results		
					No. of positive	* S/P mean for positive	%
1	Giza	BP	32	23	19	1.023	83
2	Ismailia	BP	38	25	20	0.980	80
3	Behera	BP	28	20	14	0.850	70
4	Behera	BP	26	20	15	0.905	75
5	Kaliobia	L	36	22	0	-	0
6	Giza	L	35	25	0	-	0
7	Behera	BP	35	25	0	-	0
8	Behera	BP	30	30	0	-	0
9	Behera	BP	40	30	21	0.845	70
10	Dakahlia	BP	32	25	18	0.966	72
Total				245	107		43.7

*S/P ratio greater than 0.6 indicated presence of ALV-J antibodies.

Results of ALV-J DNA detection in tissues using PCR test:

PCR test conducted on DNA extracted from tissues showed tumor lesions from the tested flocks. PCR test revealed positive amplification of 545 bp fragment with the extracted DNA of ALV-J in lanes (4, 5, 6, and 7) and negative amplification in lanes (8 and 9) for 2 flocks as shown in photo (1). Four broiler parent flocks were positive PCR test out of six flocks positive for ELISA test as shown in table (5).

Table (5): Results of PCR test for detection DNA of ALV-J in tissues:

No.	Governorate	Age	Type	PCR results
1	Giza	32	BP	+
2	Ismailia	38	BP	+
3	Behera	40	BP	+
4	Dakahlia	32	BP	+
5	Behera	35	BP	-
6	Giza	35	L	-

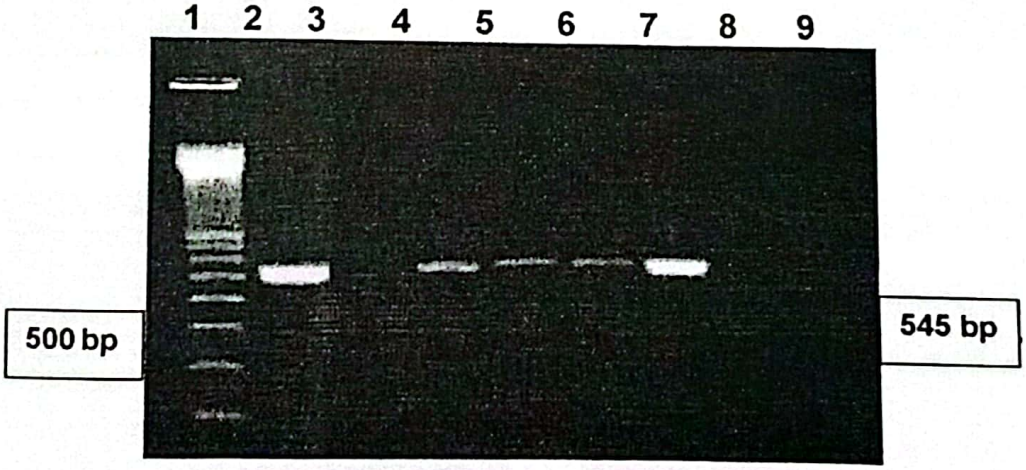

Photo (1): Electrophoresis of PCR on 1.5% agarose gel stained with ethidium bromide showing:

* 100 bp molecular weight ladder (lane 1).
* Positive control amplified DNA products at 545 bp (lane 2).
* Negative control (lane 3).
* Amplification products at 545 bp fragment indicating DNA of ALV - J with H5, H7 primers (lanes 4, 5, 6 and 7).
* Samples (lanes 8, 9) indicating negative amplification.

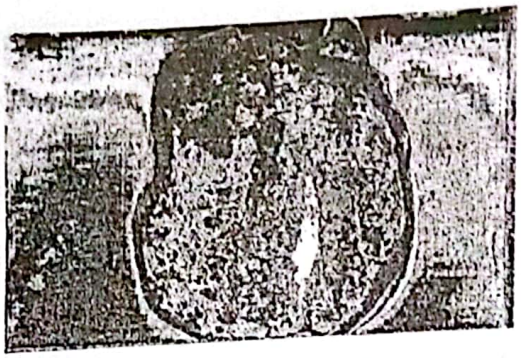

Fig. (1): Liver showing diffuse enlargement with white creamy nodules on its surface.

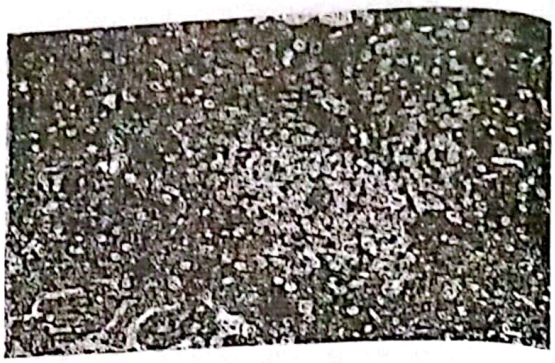

Fig.(2): Liver showing perivascular myelocytic aggregation (H & E x400).

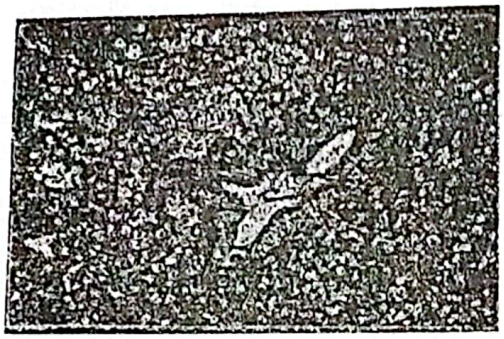

Fig. (3): Liver showing hyperplasia of bile duct surrounded by myelocytic cells (H&Ex400).

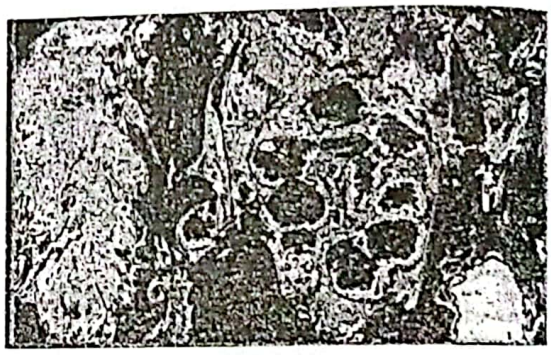

Fig. (4): Kidney showing diffuse enlargement with greyish white patches and subcapsular hemorrhages.

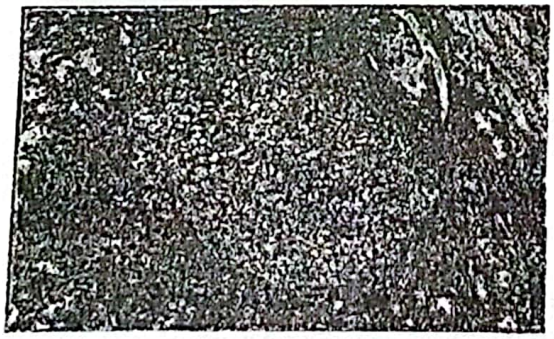

Fig. (5): Kidney showing focal aggregation of myelocytes surrounding dilated blood vessels (H&Ex100).

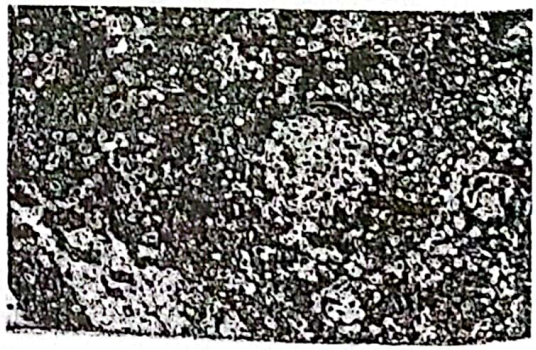

Fig. (6): Kidney showing intra-tubular cellular cast composed mainly of myelocytes and epithelial cells (H&E x 200).

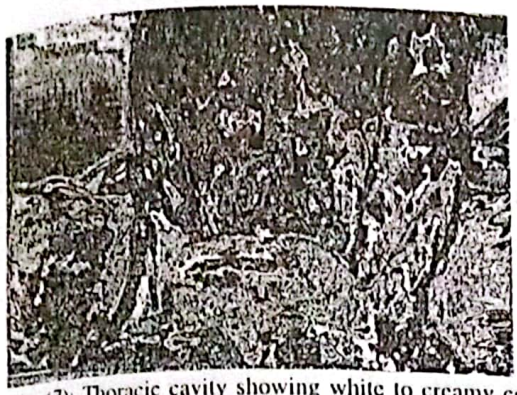

Fig. (7): Thoracic cavity showing white to creamy color nodules on the inner surface of sternum and heart.

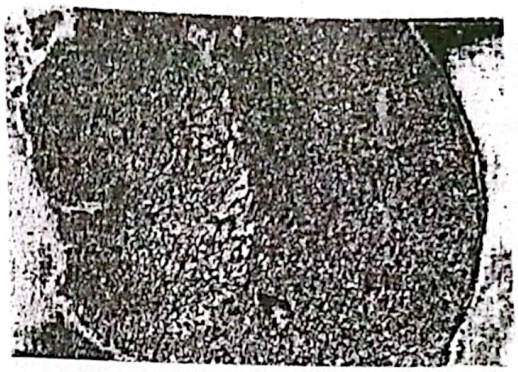

Fig. (8): Heart showing diffuse aggregation of myelocytic cells (H&E X 40).

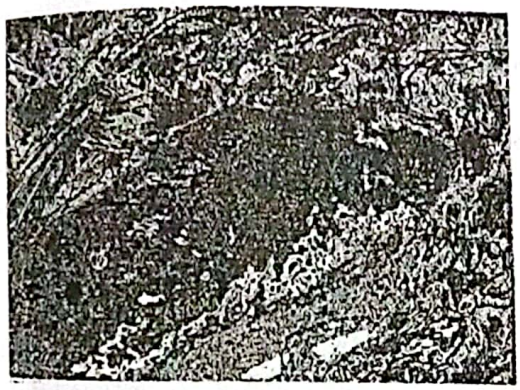

Fig. (9): Skeletal muscle showing multiple greyish white nodules

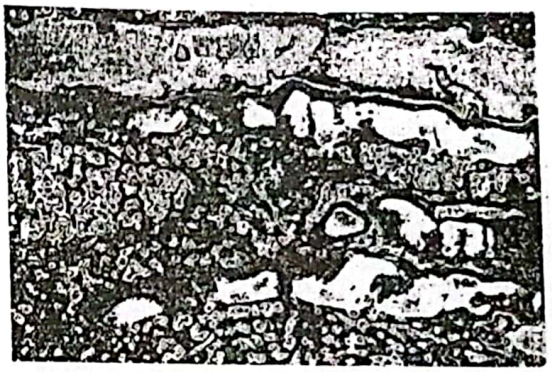

Fig. (10): Skeletal muscle showing Zenker's necrosis and myelocytes infiltration (H&E X 400).

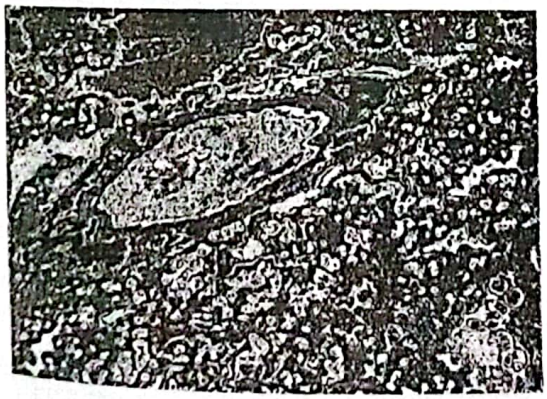

Fig. (11): Sternum section showing diffuse infiltration of myelocytes around degenerated cartilage (H&E X 400).

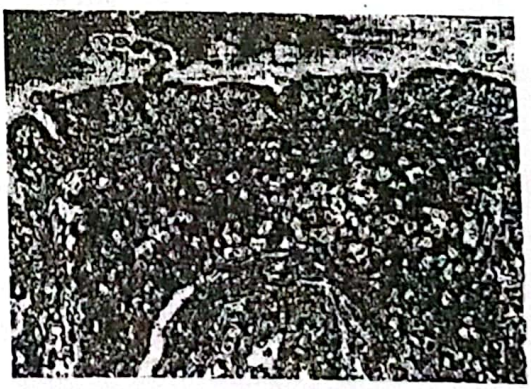

Fig. (12): Intestinal mucosa showing massive infiltration of myelocytes (H&E X 200).

Fig. (13): Abdominal cavity showing diffuse enlargement of kidney lobules and ovarian atrophy.

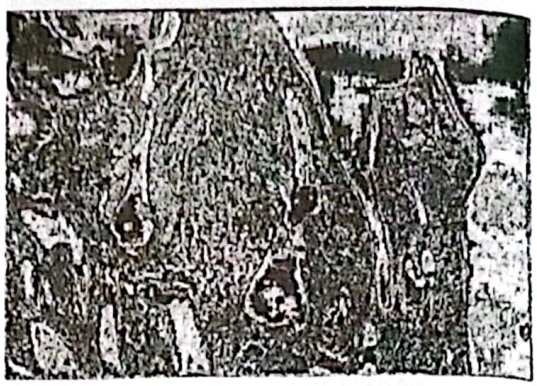

Fig. (14): Ovary showing atrophy of its ovarian follicles with focal aggregation of myelocytes (H&E X 100).

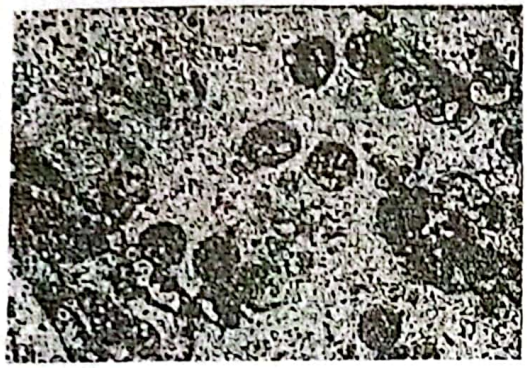

Fig. (15): Ovary showing myelocytes with brilliant red cytoplasmic granules stained with May Grunwald Gimsa (X 400).

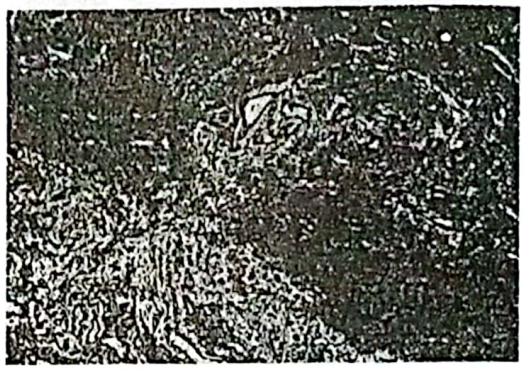

Fig. (16): Nephroblastoma showing herniation of tumor through thick fibrous connective tissue to healthy part (H&E X 200).

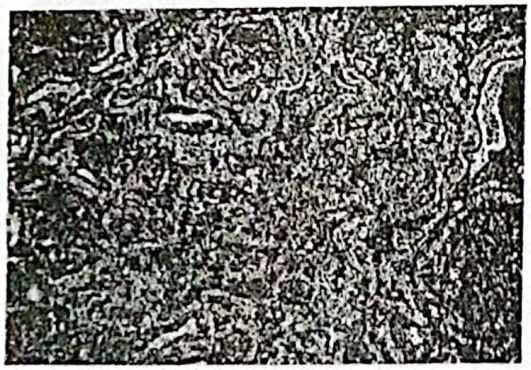

Fig. (17): Nephroblastoma showing undifferentiated basophilic tubular epithelial lining (H& EX 200).

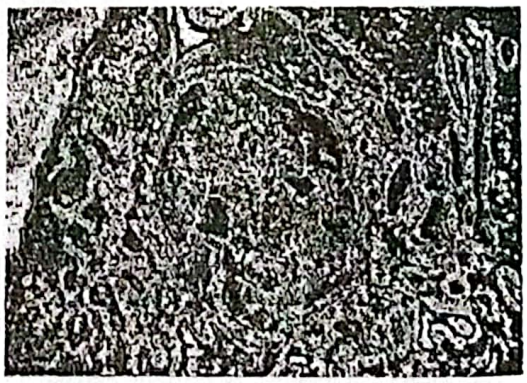

Fig. (18): Nephroblastoma showing primitive glomerulu (H&E X 200).

DISCUSSION

The present work was designated for diagnosis of avian leukosis virus subgroup J in both layers and broiler parents. The investigations of infected chicken flocks based on pathological, serological, and molecular biological methods.

Myeloid leukosis was associated with the infection of a novel subgroup of ALV designated J in meat type chickens in United Kingdom as firstly recorded by Payne et al. (1991). On the other hand ALV-J was recorded in broiler breeder flocks in Egypt by Mona, (2000).

Myeloid leukosis virus spreads vertically and horizontally leading to severe damage in the poultry industry. Recently, the sequence changes of env gene in variable regions caused rapid variation of the antigenicity of ALV-J leading to emerging of variant viruses due to the antigenic variation as elucidated by Venugopal et al. (1998).

The transforming strains of ALV that induce myelocytomatosis, such as MC29 and CMII, which carry the v-myc oncogene was reported by Enrietto and Hayman (1987). Slowly transforming strains of ALV subgroup- J that also induce myelocytomatosis, such as HPRS-103 and ADOL-Hc1, do not carry an oncogene, but molecular studies of HPRS-103 that induced myelocytomatosis indicated that c-myc was activated (Chesters et al.,2001). The acutely transforming strain 966 ALV, derived from myelocytoma and induced by strain HPRS-103 of subgroup- J ALV, has been shown to carry v-myc as reported by Payne et al. (1993).

Arshad et al. (1997) concluded that HPRS-103 showed a lower propensity to replicate in the medullary region of the lymphoid follicles of fabricius bursa more than RAV-1 strain of subgroup-A avian leukosis virus. This low bursal tropism may be a factor in why HPRS-103 did not induce lymphoid leukosis. This strain of subgroup-J replicated in blood monocytes cultures from chickens indicating a tropism for the myelomonocytic cell linage.

Studies on HPRS-103 and 966 showed that they have a tropism for the myelomonocytic cell lineage rather than the lymphoid cell lineage, which may related to their ability to cause myelocytomas as reported by Arshad et al., (1999).

Diagnosis of avian leukosis virus subgroup-J in commercial brown egg layers in China was based on observations of gross lesions, histopathology, and PCR tests. The affected birds showed yellowish/ white tumors which were observed on the visceral surface of the sternum in nodular form Binrui Xu et al. (2004).

Our serological diagnosis of suspected birds by using ELISA test revealed that 6 flocks were

positive for ALV-J antibodies by different percentage and titer. Presence of ALV-J antibodies indicated that, these flocks exposed either to the current or past infection. The rest 4 flocks were negative for ALv-J antibodies. This serological method was recommended by Venugopal (1999).

Detection of ALV-J DNA in tissues (liver, kidney, and ovary) of infected birds was applied by using PCR test. The results of polymerase chain reaction gave the confirmation to the results of histopathology for myeloid leukosis. It gave positive amplifications at 545 bp to the 4 flocks. These results coincide and came parallel with the data reported by Smith et al. (1998), and Binrui et al. (2004).

The first alterations of ALV subgroup- J occurred in bone marrow, which characterized by overcrowding of intra-sinusoidal spaces with two types of cells, the myeloid stem cell and the neoplastic myelocytes. The latter appears to arise directly from the stem cell and differentiation is arrested both non-granulated and granulated myelocytes. Tumors formed by expansion of marrow growth and may crowd through the bone and periosteum. Extramedullary tumors may also arise by blood-borne metastasis as stated by Payne and Fadly (1997).

Our results revealed that myeloid tumours, grossly appeared as dull, grayish white or creamy white, friable to cheesy and nodular or diffuse in manner. These findings come in agreement with Payne et al. (1991).

Histopathologically, myeloid tumours consisted of uniformly arranged myelocytes with very little stroma so, the myeloid tumours are characterized by high cellular and less stromal connective tissue.

The myelocytic cells characterized by large vesicular eccentrically located nucleus. The cytoplasm was tightly packed with eosinophilic granules which were usually spherical. When these cells stained by May- Grünwald- Gimsa the granules appeared brilliant red or purple. These findings come in agreement with that reported by Mladenov et al. (1967).

Livers of infected birds were slightly swollen with mottled grayish/white pinpoint spots under the capsule. Spleens were enlarged either slightly or may reach to several times of normal and had yellowish/ white tumor nodules in a few cases. Kidneys were markedly swollen, and some had light grayish/ white mottled tumor masses. Ovaries and oviducts of hens were undeveloped at 170 days of age. These findings were agreed with Sultan et al. (2004).

Myelocytic cells were aggregated near blood vessels and portal triads then invaded the hepatic cords. Also; myelocytes were replacing the hepatocytes leads to degenerative changes and atrophy of the hepatic cells. The principal

phenomena of this pattern are the formation of invasive growth in the parenchymatous organs. These findings are similar to that reported by Beard, (1980).

Tumor cells were present around veins and arteries in the liver and grew focally. In the spleen, lymphocytes decreased and tumor cells were widely present in both the red pulp and the white pulp. Epithelial cells of renal tubules were swollen, degenerated and separated from the basement membrane. Proliferated tumor cells grew focally in the ovary and oviduct. Few myeloid tumors were observed in the myocardium. These findings coincide with that observed by Binrui et al. (2004).

Myeloid tumors were seen in periosteum and near the cartilages, therefore any adjacent tissue or organ may be affected. Tumors often developed at the costochondral junction of the ribs and inner sternum. Flat bones of the pelvis and synsacrum were also affected. These findings coincided with Payne et al. (1991) and Payne & Fadly (1997).

Avian lecukosis virus subgroup-J was recorded in white Leghorn egg layer flocks being used to produce fertile eggs for human vaccine production exhibited dramatically in low egg production and high number of non- laying birds after the onset of sexual maturity. Gross lesions of freshly dead birds necropsied revealed lacking ovarian activity approximately 60% and had lesions of bacterial bursitis or synovitis, whereas the other 40% had tumors of the viscera but not of the bursa of fabricius Gingerich et al. (2002). They also suggested that hatching of day-old egg type chicks with ALV-J infected meat-type chicks in a common hatchery had contributed to cross infection.

Nephroblastoma is considered as embryonal highly malignant tumour. This type of tumour was recorded in two examined birds infected with ALV-J. The kidneys of infected birds showed spherical, well demarcated tumor mass that replaced part of the parenchyma. On cut section, the tumor appeared soft, friable, grayish white with haemorrhagic spots.

Microscopically, the tumor mass was separated from apparently healthy tissue by thick fibrous connective tissue and leukocytic infiltration. Structures like glomeruli or metanephric precursor of glomeruli were observed. The interstitial tissue showed polymorphic stromal cells which appeared round or stellate in shape. The renal tubules were lined by cuboidal or columnar epithelium with hyperchromatic nuclei. The epithelium of the renal tubule was merged with stromal cells. This finding comes in accordance with Payne et al. (1993).

This study concluded that the histopathological examination plays a crucial and decisive role in diagnosis among different types of neoplasm

caused by avian leukosis virus due to different target tissue affections. Application of specific stains for granular myelocytes was aid for differentiation between the lymphoid and myeloid leukosis.

REFERENCES

Arshad, S. S.; Howes, K.; Barron, G. S.; Smith, L. M.; Russell P.H. and Payne L. N. (1997): Tissue tropism of the HPRS-103 strain of J subgroup avian leukosis virus and of a derivative acutely transforming virus. Vet. Pathol. 34:127-137.

Arshad, S. S.; Smith, L. M. ; Howes, K.; Russell, P. H.; Venugopal, K. and Payne L. N. (1999): Tropism of subgroup J avian leukosis virus as detected by in situ hybridization. Avian Pathol 28:163-169.

Bancroft, J.D. and Cook, H.C. (1993): Manual of histological techniques. 1st ed. Churchill Livingstone, London and NewYork.

Beard, J. W. (1980): Biology of avian oncornaviruses. In G. Klein (ed.) Viral Oncology Raven Press: New York, 55-87.

Binrui Xu, Weixing D.,Chunming Y., Zhaoqing H., Yanli L., Yanzheng, S.; Xiaoyu, P.; Ning, L.; Lee, L. P. and Maoxiang L. (2004): Occurence of avian leukosis virus subgroup J in commercial layer flocks in China. Avian Pathol. 33: 13-17.

Chesters, P.; Howes, K.; McKay, J. C.; Payne, L. N. and Venugopal K. (2001): Acutely transforming avian leukosis virus subgroup J strain 966: Defective genome encodes a 72-kDa gag-myc fusion protein. J. Virol. 75:4219- 4225.

El Ghoary, A.A.; Sultan, H.A.; El-Sawy Asia, M. and Ahmed, A.S. (2000):Sero-epidemiological, haematological and Histopathological studies on tumor virus infection in broilers and commercial brown layer chickens. Vet. Med. J., Giza,48 , (4) : 539-555.

Enrietto, P. J. and Hayman, M. J. (1987): Structure and virus associated oncogenes of avian sarcoma and leukemia viruses. In G. F. de Boer (ed.). Avian Leukosis Martinus Nijhoff: Boston, MA, 29- 46.

Fuchs, A.; Myerick, B.; Jackson, T. and Leathers, V. (2000): Development of an avian leukosis virus subgroup J specific antibody test kit. In: International symposium on ALV-J and other Avian Reteroviruses Rauischolzhausen, Germany, 5-8 June 2000.

Gingerich, E.; Porter, R.E.; Lupaiani, B. and Fadly, A.M. (2002): Diagnosis of myeloid leukosis induced by recombinant avian leukosis virus in commercial; white Leghorn egg laying flocks. Avian Dis., 46 (3):745-748.

Mladenov, Z.; Heine, U.; Beard, D. and Beard, J. W. (1967):Strain MC29 avian leukosis virus. Myelocytoma, endothelioma, and renal growths: Pathomorphological and ultrastructural aspects. J. Natl. Cancer Inst. 38:251-285.

Mona, A (2000): Isolation of subgroupñJ like avian leukosis virus associated with myeloid leukosis in meat- type chickens in Egypt. In: International symposium on ALV-J and other avian reteroviruses. Rauischolzhausen, Germany, 5-8 June 2000.

Murray, M.G., and Thompson, W.F. (1980): Rapid of high molecular weight DNA, Nucleic Acids Res., 8, 4321.

Nakamura, K.; Ogiso, M.; Tsukamoto, K.; Hamazaki, N.; Hihara, H. and Yuasa, N. (2000): Lesions of bone and bone marrow in myeloid leukosis occurring naturally in

adult broiler breeders. Avian Dis., 44:215-221.

'ayne, L. N.; Brown, S. R.; Bumstead, N.; Howes, K.; Frazier, J. A and Thouless, M. E. (1991): A novel subgroup of exogenous avian leukosis virus in chickens. J. Gen. Virol. 72:801-807.

'ayne, L. N.; Gillespie, A. M. and Howes, K. (1992): Myeloid leukaemogenicity and transmission of the HPRS-103 strain of avian leukosis virus. Leukemia 6:1167-1176.

'ayne, L. N.; Gillespie, A. M. and Howes K. (1993): Recovery of acutely transforming viruses from myeloid leukosis induced by the HPRS-103 strain of avian leukosis virus.Avian Dis. 37:438 - 450.

'ayne, L. N. and Fadly, A. M. (1997): Leukosis / Sarcoma group. In: Diseases of poultry, 10th Ed. B. W.Calnek, H.J.Barnes, C.W.Beard, L.R.McDouglald, and Y.M.Saif, (eds).Iowa State Univ. Press: Ames, IA, 414-466.

'ayne, L.N. (1998): HPRS-103; a reterovirus strikes back the emergence of subgroup J avian leukosis virus. Avian Pathol., 27:36-45.

Regenmortel, M. H. V.; Fauquet, C. M.; Bishop, D. H. L.; Carstens, E. B.; Estes, M. K.; Lemon, S. M.; Maniloff, J.; Mayo, M. A.; McGeoch, D. J.; Pringle, C. R. and Wickner, R. B. (eds.) (2000): Virus Taxonomy Classification and Nomenclature of Viruses. Academic Press: New York, 1162.

Smith, L. M.; Brown, S. R.; Howes, K.; McLeod, S.; Arshad, S. S.; Barron, G. S.; Venugopal, K.; McKay, J. C. and Payne, L. N. (1998): Development and application of polymerase chain reaction (PCR) tests for the detection of subgroup J avian leukosis virus. Virus Research 54:87-98.

Sultan, H.A.; El-Sawy Asia M. and Ahmed, A.S. (2004): Avian leukosis virus subgroup- J (ALV-J) Infection in commercial white layer chickens. Proceedings of 6th Sscientific Conference of the Egyptian Veterinary Poultry Association. Sep.25-27th: 89-99.

Venugopal, K.; Howes, K.; Barron, G. S. and Payne, L. N. (1997): Recombinant env-gp85 of HPRS-103 (subgroup J) avian leukosis virus: antigenic characteristics and usefulness as a diagnostic reagent. Avian Dis., 41:283-288.

Venugopal, K.; Smith, L. M.; Howes, K. and Payne, L. N. (1998): Antigenic variants of J subgroup avian leukosis virus: sequence analysis reveals multiple changes in the *env* gene. J. Gen. Virol. 79:757-766.

Venugopal, K. (1999): Review on Avian leukosis virus subgroup J:a rapidly evolving group of oncogenic reteroviruses. Research Vet. Science. 67 :183- 119.

Vet.Med.J.,Giza. Vol.54, No.3. (2006):579-593.

COMPARATIVE STUDY ON INDOOR AIR QUALITY BETWEEN CLOSED AND OPEN BROILER ENVIRONMENTS DURING WINTER IN EASTERN REGION, KSA

ZAKIA .A. M. AHMED ; ZAHRAA.H.ALGHAMDI and MASHAEL . M. AL-NAMSHAN
Zoology Department ,Girls College Science , Dammam , Eastern Region , KSA

Received: 10.2.2006
Accepted: 9. 3. 2006

SUMMARY

The current field study was applied on two available different broiler environments (closed and open houses) during winter season in two different localities, AlJuaymah (NE) and UmSahik (NW) to Al Dammam city. A total of 20500 and 6850 birds of Rose-308 and Cobb-500 breeds respectively were used to study the effect of different broiler environments on their indoor air quality and the microbial ecology of air and litter started day before baby chicks admission up till marketing. The indoor air parameters included (Ta.C°, RH % ,AV m/sec) , some gases (CO_2 and NH_3 ppm) and microbial load of air and litter (fungal and bacterial colony forming units, cfu counts/m^3 and cfu/ gm respectively).The results revealed the following:- During winter season the closed system seemed to be more suitable for brooding baby chicks regarding to controlled indoor Ta C°, RH % and AV m/sec, despite the expected gases accumulation for keeping warm environment and increased litter microbial load and air fungal load that represent risk factors for both birds and their keeper .The obvious effect of indoor air parameters (positive correlation except CO_2 showed negative one) and litter microclimate on microbial loads in both environments threw light on efforts must be done by owners and ever alerts to follow up , manage and alternate the indoor conditions for controlling indoor microbial niches , starting before chicks admission till marketing to keep indoor and outdoor livings health Open system characterized by significantly low ered indoor air parameters levels Vs closed sys tem Indoor Ta .C° showed positive correlatio with litter f cfu only , While RH %, CO_2 an NH_3 were positively correlated with air and litt microbial load.

INTRODUCTION

The raising poultry in confinement houses dev oped from an economic need for high producti

yield utilizing little space and the consequent concentration of their waste products and contaminants mainly gases (Jones et al ; 1984). The concentration of ammonia was differed between sites in the rate of release from the litter as well as the seasonal variations, where it was increased in winter and with age 12-45 ppm Vs summer 2-9 ppm that might be attributed to the lower ventilation rates (ConceiCao et al; 1989 and Redwine et al; 2002). Litter moisture, pH, temperature and ionized ammonia ($NH4^+$) contributed to NH_3 volatilization from litter surface, where the mechanically ventilated houses could be easily monitored than naturally ventilated because of its accumulation near the litter for floor-raised bird and near the air exhaust (Gates et al 2000;NAS, 2002 and Wheeler et al ; 2003). Airborne microorganisms might be liberated directly into the air (fungi, bacteria and viruses) and could transmit for long distances by way of ventilation system into the environment depending upon kinds of microbes, location and the environmental conditions (humidity, temperature) of the samples taken that in turn might affect the respiratory health of people living close to livestock (Theresa and Wathes, 1989 ; Al-Dagal and Daniel 1990; Hartung .1994 and Zucker et al 2000). Winter air in turkey confinement houses contained significantly higher concentration of some fungi and yeast species Vs summer air (Debey et al , 1995) . Ventilation is used to remove noxious gases including ammonia and carbon dioxide as well as moisture in building so altering the microbial ecology water damaged sites (Wayon , 2004 and Nevalainen and Seuri 2005) . Therefore, the current study was carried out to throw light on the effects of different broiler environments on their indoor air quality including temperature ,relative humidity ,air velocity, some gases as NH_3 and CO_2 and the effects of these parameters on the microbial ecology (fungal and bacterial colony forming units) in air and litter.

MATERIALS AND METHODS

a) Site description:

The current field study was applied on two available different broiler environments (closed and open houses) during winter season in AlJuaymah (NE) and UmSahik (NW) localities respectively to Dammam city,KSA

b) Procedure:

Total 6 and 5 available visits (weekly) were done in accordance started day before baby chicks admission up till marketing A total of 20500 and 6850 birds of Rose-308 and Cobb-500 breeds respectively started from day before baby chicks receiving up till marketing .The indoor air parameters were measured and recorded on field (Ta.C RH %) using digital thermo hygrometer and A (m/sec) using anemometer, some gases (CO_2 a

NH_3 ppm) using Kitagawa precision gas detectors (Komyo) pump and specific detecting tubes for each gas (NO .126 SF and NO 105 SD tubes respectively) according to(Lott et al;1998 and Bruzual et al ; 2000). Air microbial loads (f and b cfu counts/m≥) were estimated gravitationally by exposed open plates contained nutrient and sabaroud agar (2 plates from each media/ site/ visit) were located in six fixed sites represented all indoor air volume occupied the house for 15 minutes each (Sauter et al ;1981). The well defined labeled collected air sampled plated were kept in portable fridge (cooler) till back to the college lab. where they were incubated either at 37°C /24 hours (bacterial growth) or 24-37°C / 24-48 hours (fungal growth).The total colony forming units (cfu) were counted used manual colony counter (mini light box, Bel-Art product NO. 37862-0000).The collected data of indoor air parameters and microbial viable counts were subjected to statistical analysis using personal Spss V 10 to get X±SD , correlations (r) and T-test values.

RESULTS AND DISCUSSION

Results in table- 1 showed in closed system, mean values of indoor Ta. C° was 27.94C°± 2.054 & RH% was 57.20± 8.377 and AV was 0.335±0.445 m/sec. The lowered temperature during brooding especially 1st week , less than recommended 34 C° by (Sainsbury , 2000) reflected the efforts should be done during winter to keep required environmental temperature despite the heat control used in this system ,while CO_2 was 903.0±511.0 and NH_3 was 10.179±10.807. These levels looked high and annoying birds (noticed difficult breathing, gasping, collected birds near doors during workers activities) , their keeper and even the researchers which was as a characteristic field feature also as a consequence of reducing ventilation rate to save fuel cost for warming during brooding, this was coincided with explanation of (Bottje et al;1998) especially for ammonia and because man and chicks supposed to be sensitized by level started 5 ppm (Tom Tabler, 2003). Mean indoor microbial load (Table-2) for air f.cfu was $45.35x10^3$±40.48 & air b.cfu was 142.50 $x10^3$ ± 217.00 while litter f.cfu was 614.66 $x10^3$ ± 5261.29 & litter b.cfu was 9743.35 $x10^3$ ± 581.47. Litter had higher microbial load Vs air and the higher loads were at 35 days old for all except air f.cfu was at 28 days old, the association of increased ammonia levels on 21-28 days old with the increased fungal count in air confirmed inadequate ventilation that enclosed the indoor gases not exhaled. On regarding the effect of indoor air parameters on microbial ecology shown in (Table 3), the indoor Ta.°C was positively correlated with air microbial load f & b (P= 0.042 & 0.002 respectively) but negatively correlated with

litter f & b (p= 0.109 & 0.066 respectively).Indoor RH% and AV were positively correlated with air f.cfu only (p=0.001&0.015 respectively).Meanwhile, CO_2 gas had no significant effects or correlations with indoor air but with litter f. cfu only(p=0.108), despite NH_3 showed positive correlations with indoor air f & b (P= 0.08 and 0.086) and so with litter b. cfu (P= 0.007). Fungal growth had been demonstrated to occur in broiler litter depending on various environmental factors especially litter microclimate (Schipper et al;1982 and Bacon,1985).

Open ecosystem, (Table 4) revealed that mean indoor Ta.°C was 18.49 ± 2.188 & RH% was 69.31 ± 5.89 and AV was 0.15 m/sec ± 0.087. Indoor CO_2 mean was 447.84 ppm ±105.31 and NH_3 was 3.254 ppm ± 3.32. These results threw light on the severity of cool and highly fluctuated weather on housing broiler in open ecosystem during winter and the health risk for brooded baby chicks and the effect of incomplete thermal insulation on dissipating the indoor air elements and gases to outdoor air compared to the closed system. During 2-5 weeks old advised temperatures must be 27, 24 and 21 °C respectively (Sainsbury, 2000) .The negative effect of Ta. °C on fungal load was partially coincided with results of (Debey et al ; 1995).

Results in (Table 5) clarified that, mean Indoor air f cfu was less(88.20 x10 ≥± 84.42) than litter f cfu (118.94x 10 ≥ ± 251.3) while air b cfu was higher (335.2 x10≥ ± 270.6) than litter b.cfu (147.77x10≥ ± 199.75) ,these differences should be considered regarding the effect of both indoor air and litter microclimate on kind microbial ecology which also related to kind environment.

Data in (Table 6), Indoor Ta .°C showed positive correlation with litter f (p=0.017) and so RH% with air f & b (p=0.039 & 0.012 respectively)..AV had no significant correlation with indoor air and litter microbial loads that might be attributed to the improper and low Av Vs closed system, so air circulation and redistribution of microbial loads were not recognizable between air and litter. Indoor CO_2 was positively correlated with air f & b (p=0.008 & 0.046 respectively) as well as with litter f (p=0.025).On the other hand NH_3 was positively correlated with air f & b (p= 0.00 for both) and with litter f & b (p= 0.002 & 0.00 respectively).These findings might be attributed to the possibility of dispersed contaminated food particles with fungi accompanied humid environment .Ammonia gas generation and emission were mostly result of litter microbial activity and interaction of indoor climatic factors (Weaver and Meijerhof; 1991 and Groot-Koerkamp, 1994). The effect of indoor RH% on air microbial population ecology confirmed by (Al-Dagal and Daniel,1990). The positive correlations between indoor gases and microbial population were

recognized partially as their metabolites and partially birds exhaled air (Gustafasson and Martensson , 1990) , On comparing the mean differences of indoor climate between closed and open environments as shown in (table 7) showed significant mean differences were in indoor Ta .°C , AVm/sec, CO_2 and NH_3 ppm where increased in closed Vs open (p= 0.001, 0.036 ,0.001 and 0.001 respectively) while RH% increased in open Vs closed (p=0.001), the effect of season on indoor gases accumulation (mainly ammonia) especially in closed Vs open houses was previously confirmed by (Seedorf and Hartung , 1999) . The nature of environment affected some of indoor microbial loads as revealed in (table 8) where indoor air f & b cfu were significantly increased in open Vs closed (p=0.033 and 0.008 respectively) , while in closed environment the litter b.cfu were significantly increased Vs open(p=0.018) . From the aforementioned results it could be concluded that closed system had high indoor gases levels that might annoying birds and their keeper. Litter had higher microbial load Vs air and the higher loads were at 35 days old for all except air f.cfu was at 28 days old , the association of increased ammonia levels on 21-28 days old with the increased fungal count in air confirmed inadequate ventilation rates that enclosed the indoor gases not exhaled .The indoor Ta. °C was positively correlated with air microbial loads(f & b cfu counts) but negatively with that of litter. Indoor RH% and AV were positively correlated with air f.cfu, CO_2 gas had no significant effects or correlations with indoor air loads but found with litter f cfu . Meanwhile, NH_3 showed positive correlations with indoor air f & b and so with litter b cfu. Open system had lowered indoor air parameters than closed . Indoor air f. cfu mean was less than in litter .These differences should be considered regarding the effect of both indoor air and litter microclimate on microbial ecology. Indoor Ta .°C showed positive correlation with litter f. cfu , RH%, CO_2 and NH_3 were positively correlated with air f & b cfu as well as with litter f. cfu with CO_2 . Significant mean differences were noticed between environments , indoor Ta, AV, CO_2 and NH_3 were increased in closed Vs open, while RH% increased in open Vs closed . Conclusively, the effect of season on indoor gases accumulation (mainly ammonia) was noticeable in closed Vs open house .The nature of environment affected the indoor microbial ecology, where indoor air f & b. cfu were significantly increased in open Vs closed in closed environment the litter b.cfu were significantly increased Vs open. The severity of cool and highly fluctuated weather on housing broiler in open ecosystem during winter should be considered as health risk for brooded chicks and the effect of incomplete thermal insulation on dissipation of indoor air gases to outdoor air.

Table 1: Mean values (X±SD) of indoor air parameters in closed ecosystem during winter.

Air parameters	V (1)		V (2)		V (3)		V (4)		V (5)		V (6)		Visits (total)	
	X	±SD	X	±SD	X	±SD	X	±SD	X	±SD	X	±SD	X	±SD
Ta. .C^0	26.2750	.1500	30.2200	1.7297	29.0800	1.0918	28.5400	.5771	28.2800	1.3027	25.0400	1.1480	27.9621	2.0545
RH. %	47.1250	1.4175	60.0800	8.6439	57.1600	4.4948	67.2000	2.6306	61.7800	3.1586	48.0800	3.3071	57.2414	8.3778
A.V. (m/sec)	.25000	.17321	.19400	012280	.48000	.68702	.68000	.77910	.18000	.08367	.21000	.08944	.33517	.44534
CO2. ppm	324.7500	16.500	490.000	74.1620	800.0000	187.0829	1720.000	311.4482	1340.000	89.4427	860.0000	245.9675	943.0690	511.4117
NH3. ppm	.1300	.01414	.2800	.1903	4.4000	1.5166	16.2000	6.3008	27.6000	8.1425	13.0000	4.4721	10.6179	10.8679

Ta . , RH and AV = ambient temperature .C° & relative humidity % and air velocity in meter/second(m/sec).
CO2 and NH3 .= carbon dioxide and ammonia in part/million(ppm).
V= visit number.

(Table-2):Mean values(X±SD) of microbial loads of indoor air and litter in closed ecosystem during winter.

Air & litter	V (1)		V (2)		V (3)		V (4)		V (5)		V (6)		Visits (total)	
	X	±SD	X	±SD	X	±SD	X	±SD	X	±SD	X	±SD	X	±SD
air –f	5.50	5.43	50.74	28.10	46.32	47.02	52.93	41.87	95.84	21.22	12.78	5.85	45.35	40.48
air –b	3.18	3.28	50.34	31.79	12.42	0.26	92.14	62.30	146.01	93.42	523.01	284.01	142.50	217.00
litter –f	0.03	0.002	59.22	48.29	32.92	40.26	177.82	370.45	81.84	71.92	309.74	502.33	6114.06	5261.29
litter –b	2.67	2.04	285.74	329.24	97.20	52.11	73.24	64.03	859.60	597.52	1196.45	650.71	9743.35	581.47

V= visit number F= fungal load B= bacterial load

(Table 3);Effect of indoor air parameters on air and litter microbial loads in closed ecosystem during winter.

Air parameters	Air - F	Air – B	Litter-F	Litter-B
Ta.C°	.380** .042	-.555*** .002	-.304* .109	-.346* .066
RH%	.627*** .001	-.254 .185	-.037 .851	-.187 .332
A.V (m/sec)	.447*** .015	-.128 .507	.113 .560	-.168 .383
CO2ppm	.286 .133	.135 .486	.304* .108	.134 .490
NH3ppm	.485*** .008	.324* .086	.209 .277	.493*** .007

. (Table-4);Mean values(X±SD) of indoor air parameters in open ecosystem during winter.

Air parameters	V (1)		V (2)		V (3)		V (4)		V (5)		Visits (total)	
	X	±SD	X	±SD	X	±SD	X	±SD	X	±SD	X	±SD
Ta. C^0	17.7667	.3055	18.5400	.4037	21.2200	.1643	17.7600	1.7925	16.7800	2.9778	18.4696	2.1887
RH.%	56.6667	.4933	67.9600	.2702	74.8000	2.1679	73.1600	2.1373	68.9200	.7596	69.3130	5.8559
A.V. (m/sec)	.26667	.15275	.16000	.08944	.12000	.04472	.11000	.05477	.14000	.005477	.15000	.08660
CO2.ppm	353.3333	47.2582	350.0000	50.0000	470.0000	44.7214	500.0000	122.4745	528.0000	98.3362	447.8261	105.3115
NH3. ppm	.1167	.01528	.3000	.1871	1.6000	.5477	5.8400	1.7111	7.1600	2.8902	3.2543	3.3236

Ta . , RH and AV = r ambient temperature .C° & relative humidity % and air velocity in meter/second(m/sec).
CO2 and NH3 .= carbon dioxide and ammonia in part/million(ppm).
V= visit number

(Table 5):Mean values (X±SD) of indoor air and litter microbial loads in open ecosystem during winter

Air & litter	V (1)		V (2)		V (3)		V (4)		V (5)		Visits (total)	
	X	±SD	X	±SD	X	±SD	X	±SD	X	±SD	X	±SD
air –f	1.57	0.40	30.30	24.68	54.74	14.15	184.04	9360.61	135.68	95.91	88.20	84.42
air –b	3.87	1.30	5.90	6.67	357.10	134.83	555.36	217.72	568.16	174.40	335.21	270.60
litter -f	0.03	0.002	3.56	1.60	7.33	6.14	86.99	32.65	449.20	407.19	118.94	251.30
litter - b	0.18	0.13	3.60	1.69	29.32	5.51	281.72	181.38	365.00	212.70	147.77	199.75

f and b = fungal and bacterial colony forming units count.

(Table 6): Effect of indooor air parameters on air and litter microbial load in open ecosystem duringg

Air parameters	Air - F	Air – B	Litter-F	Litter-B
Ta. C^0	-.193	-.045	-.492***	-.158
	.377	.837	.017	.470
RH%	.433**	.513***	.007	.196
	.039	.012	.975	.369
A.V (m/sec)	-.291	-.256	.052	.009
	.178	.238	.813	.966
CO2ppm	.536***	.419**	.465**	.192
	.008	.046	.025	.381
NH3ppm	.759***	.822***	.607***	.854***
	.001	.001	.002	.001

Values in columns are correlation (r) and significance , * at P≤ 0.1 . ** at P ≤ and *** at P ≤. 0.001

(Table 7: Mean differences of indoor air parameters between closed and open ecosystems in winter.

Air parameters	Winter					
	Closed		Open		T	Sig
	X	±SD	X	±SD		
Ta –C	27.9621	2.0545	18.4696	2.1887	15.96	0.001***
RH.%	57.2414	8.3778	69.3130	5.8559	6.10	0.001***
A.V. m/sec	.33517	.44534	.15000	.08660	2.19	0.036**
CO2.ppm	943.0690	511.4117	447.8261	105.3115	5.08	0.001***
NH3 ppm	10.6179	10.8679	3.2543	3.3236	3.45	0.001***

Values in column are T test mean significant differences between both indoor parameters during winter.
Significance , * at P≤ 0.1 . ** at P ≤ and *** at P ≤. 0.001.

(Table): Mean differences of indoor air and litter microbial load between closed and open ecosystems in winter.

Air & litter	Winter				T	Sig
	Closed		Open			
	Mean	±SD	Mean	±SD		
air –f	45.35	40.48	88.20	84.42	2.24	0.033**
air –b	142.49	217.00	335.21	270.60	2.78	0.008** *
litter -f	6114.06	5261.30	118.94	251.30	0.07	0.946
litter -b	9743.35	58.15	147.77	199.75	2.47	0.018** *

f and b = fungal and bacterial colony forming units count.
Values in columns are correlation (r) and significance , * at P≤ 0.1 . ** at P ≤ and *** at P ≤. 0.001.

REFERENCES

Al-Dagal, M. and Daniel, Y. C. Fung. (1990): Aeromicrobiology-A review, Crit .Rev .Food. Sci. Nutr .29 (5):33-340.

Bacon, C.W. (1985): Effects of Litter Volatiles and ammonia on Fungal Spores Germination .Poult .Sci, 65:710-716.

Bottje, W .G; Wang, S; Beers, W. and Gawthon,D .(1998): Lung lining fluid antioxidants in male broiler Age-related changes under thermo neutral and cold temperature condition, Poult. Sci., 77:1905-1912.

Bruzual, J.J.; Peak,S.D Brake,J. and Peeblest, E.D.(2000): Effect of relative humidity during the last five days of incubation and brooding temperature on performance of broiler chicks from young broiler breeders.Poutl. sci., 79: 1385 - 1391.

Conceiçao ,M. A. P; Johnson, H . E. and Wathes, C.W. (1989): Air hygiene in poultry house: Spatial homogeneity of aerial pollutants . Brit. Poult. Sci, 30:765-776.

Debey, M. C; Tramplel, D. W; Richard, J.L.;Bundy, D.S; Hoffman, L. J; Meyer, V. and Mand Cox, D. F. (1995); Effects of environmental variables in turkeys confinement houses on airborne Aspergillus and mycoflora composition , Poult. Sci .74 (3);463-471.

Gates, R. S; Pestacore, A. J ; Ford, M. J ; Taraba, J. L; Liberty, K.; Cantor, A. H. and Burnham, D. J. (2000): The effect of feeding low protein diets on ammonia emission and total ammonical nitrogen in broiler litter. Proceedings of the 2000, at the poultry waste, Management .Symposium .Springdale AR. 19-21. pp: 169-180.

Groot-Koerkamp, P.W.G. .(1994): Review of emissions of ammonia from housing systems for laying hens in relation to sources , processes , building design and manure handling. J. Agri.Eng.Res, 59 : 73-87.

Gustafasson , G. and Martensson, L. (1990): Gaser och dammi fjaderfastallar . Swedish .Univ .Agri. Sci. Dept .of Farm Buildings. Report No. 68 . Lund.

Hartung, J. (1994): The effect of airborne particulates on livestock health and production , Welfare Sci . Division.55-69.

Jones, W.; Morring, K.; Olenchock, S. A.;Willioms, T. and Hickey, J. (1984): Environmental study of poultry confinement building ,Am .Ind .Hyg. Assoc. J. 45, 760.

Lott B. D.; Simmons, J. D. and May. J. D.(1998): Air velocity and air temperature effects on broiler performance. Poult. Sci., 77:391393.

NAS, National Academy of Science. (2002): Air emission from animal feeding operations : Current Knowledge, Future needs, Final report. http//www.nap.edu/books/0309087058/htm/.

Nevalainen, A; and Seuri ,M.(2005): Of microbes and men .Indoor Air , 15 (7); 58-64

Redwine, J . S; Lacey, R . E; Mukhtar, S; and Carey, J. B. (2002): Concentration and emission of ammonia and particulate matter in Tunnel-ventilated broiler houses under summer condition Texas. Am. Asoc. Agric .Eng , 45 (4): 1101-1109.

Sainsbury, D.(2000): Poultry health and Management .4th ed. Blackwell science.

Sauter, E. A; Petesen, C. F.; Parkinson, J. F.; Dixon, J. E. and Stroh, R.C.(1981) : The airborne microflora of poultry house .Poult.Sci. 60(3): 569-574.

Schippers, B.; Meijer, J. W. and Liem, J. L.(1982): Effect of soil Volatiles on germination and growth of soil fungi. Tropi .Mycol. Soc. 79:253-259.

Seedorf ,J , and Hartung, J. (1999) : Survey of ammonia concentration in livestock buildings . J. Agric .Sci , 133: 433-437.

Theresa, M. M and Wathes, C. M. (1989): Air hygiene in broiler house: Comparison of deep litter with raised netting floors .Brit .Poult. Sci, 30:23-37.

Tom Tabler .G.(2003): Brooding chicks and pullets; Environmental critical control points .Avian advices, 5 (10 : 1-5.Univ. Arkansas. Division. Agric. Cooperative Extension service.

Wayon, D. P. (2004): The effect of indoor air quality on performance and productivity. Indoor Air . 14 (10); 92-101

Weaver, W. D. and Meijerhof, R. (1991): The effect of different levels of relative humidity and air movement on litter conditions, ammonia levels, growth and carcass quality for broiler chickens Poult. Sci. 70 (4); 746-755.

Wheeler, E. F.; Zajaczkowshi, J. S.; Topper, P. A.; Gates, R. S.; Xin, H.; Casey, K. D. and Liang, Y. (2003): Ammonia emissions from broiler houses in Pennsylvania during cold weather. International. Symposium. on Gaseous and odor emissions from animal production facilities, Horsens, Jutland, Denmark. 1-4 June.

Zucker, B. A.; Trojan, S. and Muller, W. (2000): Air-borne gram-negative bacterial flora in animal houses J. Vet. Med. B. 47, 37-46.