CLINICO-PATHOLOGICAL FEATURES OF HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI-H5N1) OUTBREAKS IN COMMERCIAL CHICKENS IN NIGERIA

Kumbish, P. R.; Joannis, T. M., Jambalang, A.R; Damina, M.S; Hussaini, B.A; Akanbi, B. O.; Oyetunde, I. L; Abdu, M. H.; Danbirni, S.; James, A.; Yahaya, K; Bako; B., Nanbol, D., Elisha, I. L.; Solomon; P., Ogunsan, E. A.; Adulugba, A. P. and Okewole, P.A.

National Veterinary Research Institute, Vom, Plateau State, Nigeria

CORRESPONDING AUTHOR:
P.R. Kumbish; petersidekumbish@yahoo.co.uk
NVRI, PMB 001 Vom,
Plateau State - Nigeria

ABSTRACT:
In early January 2006, the first confirmed outbreaks of avian influenza in domestic birds occurred in commercial chicken flocks in Kaduna, Jos and Kano States in the northern part of Nigeria. The outbreaks were confirmed to be the highly pathogenic avian influenza (HPAI) caused by the subtype H5N1. The outbreak was spontaneously reported in Jos, and about 10 days later it was reported in Kano and it quickly spread to other parts of the country. The infection was characterized by very high mortality in commercial layers. It was also observed to affect turkeys, broilers, cockerels, backyard/local chickens, ostriches, pigeons, guinea fowls, geese and ducks. The gross and microscopic lesions were found to similar to what have been described earlier for the disease.

KEY WORDS: *Clinico-pathological features, commercial, chickens, highly pathogenic avian influenza, Nigeria.*

INTRODUCTION

Highly Pathogenic Avian Influenza (HPAI) is a viral disease affecting the digestive, nervous and respiratory and/or reproductive systems of all domestic and wild birds. It is characterised by high morbidity and mortality with an incubation period of few hours to 3 days. It is highly contagious and may be fatal in humans (1).

Avian influenza is caused by Influenza A viruses of the family Orthomyxoviridae and Genus Influenza A (6). Influenza A viruses are divided into subtypes on the basis of one of the 15 antigenically distinct haemagglutinin (H) antigens and nine neuraminidase (N) antigens. Influenza A viruses affecting poultry can be divided into two groups: highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) viruses on the basis of their virulence or pathogenicity. The very virulent viruses (HPAI) result in flock mortality as high as 100%, while the LPAI can cause mild disease, e.g. mild respiratory disease, depression and egg production problems in laying birds (4).

Chickens and turkeys are highly susceptible to infection and clinical disease, while ducks and geese although susceptible to infection with all AI viral strains, suffer clinical disease from only highly virulent strains. Ostriches, emus, quails, ducks, guinea fowls, pheasants, and pigeons are susceptible to AI of varying degrees of severity. Pigs and humans are equally susceptible to

infection by the HPAI virus and the potential of pigs serving as mixing vehicle for genetic re-assortment (mutation) is also reported (8). In general, AI viruses are not hardy and are susceptible to warm environmental conditions but may remain viable in cold temperatures. In farms where outbreaks have been reported, restocking could be done 21 days thereafter provided adequate and suitable decontamination of poultry houses, equipment and personnel has been carried out (8).

There has been considerable AI activity in the Eastern Hemisphere. The higher profile of AI resulting from the human infections with H5N1 and H9N2 viruses in Hong Kong, in 1997 and 1999, respectively, resulted in increased reporting and active surveillance. There have been three reported incidents of high-pathogenicity (HP) AI: H5N2 in north-eastern Italy in 1997 (eight outbreaks); H5N1 in Hong Kong in 1997 recurring in 2001 and 2002; H7N1 in north-eastern Italy resulting in 413 outbreaks in 1999-00. The Italian HPAI outbreaks were preceded by 199 H7N1 low-pathogenicity (LP) AI outbreaks in 1999, and this virus continued to cause some problems after the eradication of HPAI. During the second half of the 1990s outbreaks of LPAI due to H9N2 subtype have been reported in Germany, Italy, Ireland, South Africa, Hungary, Korea, China, Hong Kong, countries of the Middle East, Iran, and Pakistan. The continued presence of virus of this subtype in the Middle and Far East may mean it is becoming an established endemic disease in those regions. Other more restricted outbreaks in poultry have resulted in the isolation of LPAI viruses of H5, H6, H7, and H10 subtypes.

The first outbreak of HPAI in Nigeria was confirmed in Feb.2006. the disease spread like wild fire and by June, 2006, a total of 129 outbreaks had occurred in the federal Capital Territory (FCT), Anambra, Bauchi, Benue, Jigawa, Kano, Katsina, Lagos, Nasarawa, Ogun, Oyo, Plateau, Rivers, Taraba, and Yobe states of Nigeria.

In this study we described the clinical signs, gross and microscopic lesions observed in the chickens affected by some of these outbreaks caused by H5N1 subtype. The birds were submitted to the National Veterinary Research Institute, (NVRI) Vom where they were confirmed to be positive for AI. In addition we assessed the relatedness of the signs/ lesions observed in the Nigerian outbreaks with those reported in other parts of the world.

MATERIALS AND METHODS

History of the outbreak

Kaduna outbreak

The case was first reported on 16th January 2006, and was investigated on 17th January 2006. It occurred in Sambawa farm in Jaji, a small village situated along Kaduna-Kano road which has a global positioning system (GPS) of latitude 10o 48N; longitude 7o 34'E and about 20km from Kaduna city. The farm is fenced from the major highway and the surrounding households, and is about 100 hectares in size. It has a running stream with a lot of economic trees and plantation providing a haven for a variety of wild local birds. The farm had a total of 46,000 commercial layers, 200 ostriches, 200 turkeys, few geese, 4000 layer breeders, 2000 broiler chicks, about 150 heads of cattle (White Fulani) and 70 sheep (Yankasa). Death of these different poultry species were said to have started 2 weeks prior to the date of reporting. By 17th January 2006 when a team arrived the farm for investigation, over 80% of the layer flock population had died and morbidity rate was 100%.

Jos Outbreak

Similarly, on 16th January 2006 another suspected outbreak was reported from M & D farm located within residential area in Bukuru town with a GPS of 9o 47'N; longitude 8o 51'E and about 10km from Jos city. The farm had a flock size of 1000 layers kept on deep litter. There was 70% mortality within 2 days while morbidity was 100%.

Kano outbreak

By 27 January 2006, another suspected outbreak was reported in Janguza farm with a GPS of latitude 12o 0o'N, longitude 8o 3o'E in Kano metropolis. Kano (200km from Kaduna city) is in the North-western part of Nigeria and is located on same axis with Jaji where the first outbreak was detected. The farm had a flock size of 3000 layers and 2000 cockerels. It was fenced and covered a landmass of about 3 hectares and it was located within a densely populated area. The closest poultry farm is less than 1km away. There were little or no biosecurity measures put in place prior to this outbreak. Morbidity was 100% for the layers with 80% mortality while the cockerel had 30% morbidity with no mortality as at then.

Some other outbreaks were later reported in other farms in Kaduna, Jos and Kano. The disease spread fast to other States of Nigeria. As at July 2006, 15 States had reported positive outbreaks of the disease which was confirmed by the Reference Laboratory in Padova, Italy. The outbreak started in commercial laying chickens but later spread to other birds such as broilers, cockerels, ostriches, pigeons, guinea fowls, turkeys, geese, ducks and local/backyard poultry as well as wild birds.

Submission from suspected flocks

During the outbreaks, suspected cases showing severe mortality, depression, apathy,. decrease in feed and/ or water intake, diarrhoea, lack of coordination, drop in egg production e.t.c were submitted to the N.V.R.I.Vom, for post mortem examination, collection of samples for virus isolation and other laboratory analysis. The carcasses were examined immediately upon arrival..

Post mortem examination (PME)

The PME comprised macroscopic examination, especially of the head of the carcass. After removal of the skin, the abdominal cavity was open and the trachea, heart and liver were evaluated. Tissues with gross pathological changes were fixed in 10% buffered formalin for histopathological examination.

Virus Culture

Virus culture was carried out as described by (OIE, 2005). Nine days old chicken (9 day-old) embryonated eggs obtained from specific antibody negative (SAN) flocks were used for viral isolation. 0.2 ml of the supernatant (of 20% w/v tissue homogenate) was inoculated into five viable embryonated chickens eggs via the Allantoic cavity. The eggs were incubated at 37oc in a humidified incubator. They were candled daily and those with dead embryos were chilled at 4oc for 12-18 hrs, after which a qualitative analysis was carried out on the dead embryos to check for haemagglutination (HA) activity using 10% washed chicken red blood cells from SAN flocks as indicator. Allantoic fluid from eggs that showed positive for HA activity were harvested aseptically and tested for sterility by plating on blood agar and incubated at 37oc for 24 hrs (2, 6).

AGID Test

AGID test was carried out as described in OIE manual 2005. The supernatant was inactivated using 0.1% formaldehyde and used as the test antigen with standard avian influenza antigen and antisera obtained from national Veterinary Service Laboratory

(NVSL) Iowa, USA. The agar gel plates were incubated at 37oc in a humidified incubator and examined after 24 hrs. Positive specimen showed Precipitating lines of identity to avian influenza type A group specific Ribonucleic Protein (RNP) antigen in the AGID test

Haemagglutination Inhibition test (HI)

Alpha haemagglutination Inhibition test was carried with serially diluted positive Allantoic fluid using standard procedures (OIE, 2005). Newcastle disease monospecific antisera showed no inhibition of the test antigen.

RESULT

Clinical findings of the outbreaks (HPAI)

The outbreaks involved about 785,571 birds of different species (breeders, turkeys, guinea fowl, broilers, cockerels, ostriches, ducks, geese, local fowl, wild / migratory birds and pigeons) out of which 760,562 were layers. The clinical signs were variable and influenced by factors such as species affected, age, sex, concurrent diseases and environment. The clinical signs of the disease observed were depression and sleepiness or somnolence (fig 1, 2). Others were sneezing, mucus discharge from the beak and nostrils (fig 3). However, some of the typical clinical findings were seen mostly in adult chickens, namely, cyanotic combs and wattles, swollen face (fig 4).

Others are subcutaneous haemorrhages of the cervical and abdominal areas while the shanks and feet were hyperaemic and the foot pads were swollen (fig 5). Neurological signs of torticollis with unsteady gait were also observed in some (fig 6); while yellowish-green to whitish diarrhoea, severe dyspnoea and soft shell to shell-less eggs were also consistent observations (fig 7, 8).

Gross lesions

There were severe congestion and haemorrhages of nasal sinuses while the serosal and mucosal surfaces of tracheae were markedly congested. The lungs were oedematous and filled with frothy exudates (fig 9, 10) and the air sacs were cloudy. There were ecchymotic and petechial haemorrhages affecting the epicardium and inner surface of the rib cage extending down to the peritoneal cavity as well as abdominal fats (fig 11). There were diffuse haemorrhages on the serosal surface of the duodenum, jejunum, ileum and ovarian follicles (fig 14, 15, 16, 17, 18). There were varying degrees of peritonitis and the peritonitis observed in most of the cases autopsied were characterised by a low amount of fibrinous exudates and a relatively large quantity of thick white yellow coloured fluid in the abdominal cavity as against the well known peritonitis which is accompanied by a large amount of fibrinous exudates with an egg yellow colour, Fig (12, 13). The proventriculus presented ecchymotic and petechial haemorrhages particularly on the papillae (fig 19). There were different degrees of organ enlargement affecting the liver, spleen and kidneys (fig 20, 21). Generalized congestion of the musculature especially the breast and thigh muscles were consistent findings. Birds that died during the per acute stages of the disease showed little or no gross lesions.

Microscopic lesions

These were characterised by vascular disturbances leading to oedema, haemorrhages and perivascular cuffing, especially in the myocardium, spleen, lungs, brain and wattles Thus congestion of coronary vessels associated with haemorrhages and diffuse foci of richly cellular and vascular tissues containing red blood cells, heterophils, mononuclear cells and fibroblasts were evident, while there was severe mononuclear cellular infiltration of the endocardium.

There was severe hyperemia of the pulmonary arterioles and congestion of the pulmonary veins. There was evidence of pulmonary vessel damage and haemorrhage. There was thickening and inflammation of the interstitial and parabronchial wall leading to broncho-interstitial pneumonia.

Generalized congestion was seen in the central and hepatic veins with mononuclear infiltration into the hepatic septae, and lymphocytic perivascular cuffing of the hepatic vein. Loss of architecture of the hepatic cord arrangement was evident.

Proventricular glands had diffuse foci of haemorrhages and mononuclear cellular infiltration affecting both the glands and the connective tissue septae. The intestinal villi and submucosa had diffuse mononuclear cellular infiltration, while

the kidneys showed severe and generalized haemorrhages with cellular exudation in the tubular interstitial spaces.

FIG 1a &b: Dullness and somnolence

FIG 2: Showing somnolence and prostration

FIG 3: Cyanotic comb

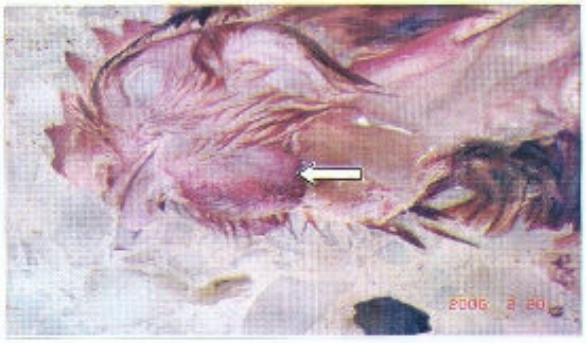


FIG 4: Cyanosis of the Wattle filled with fluid

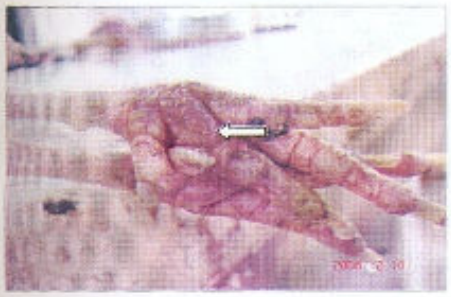


FIG 6: Torticollis

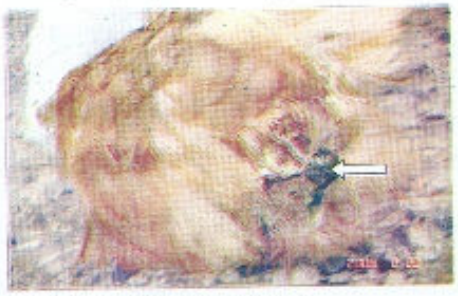
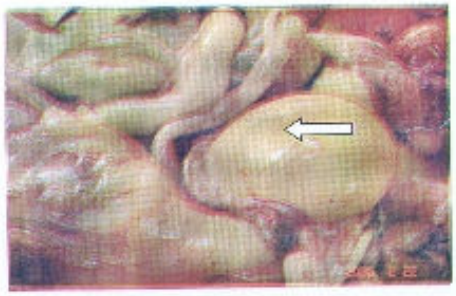


FIG 7: Yellowish-Green Diarrhoea



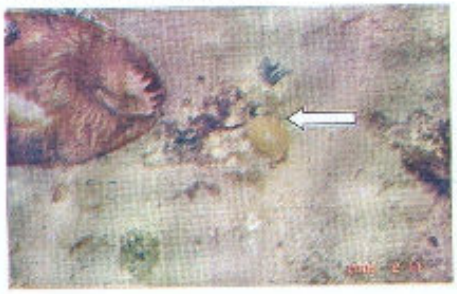


FIG 8a &b: Somnolence, Greenish-Yellowish Diarrhoea and soft shell/cracked egg

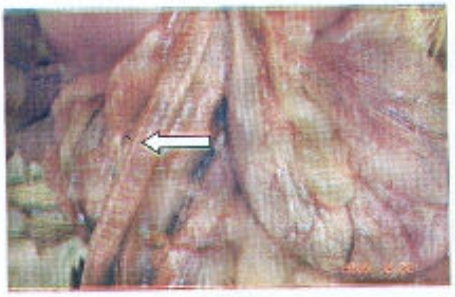


FIG 9: Hyperaemia of the trachea

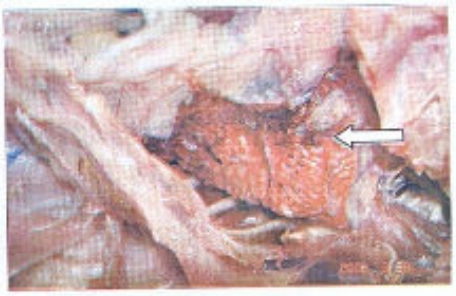
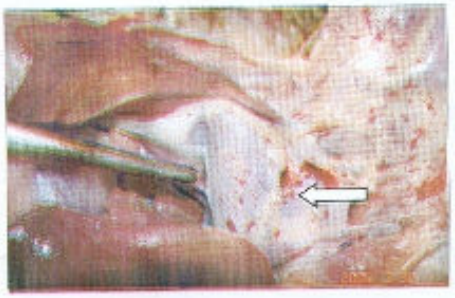


FIG 10: Congested Lung with exudates



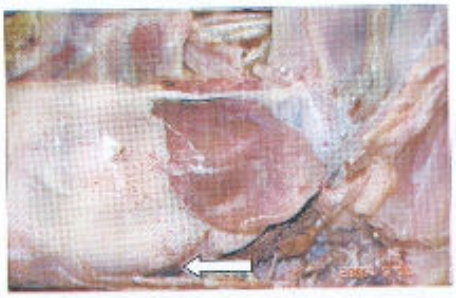


Figure 11: Petechial and ecchymotic haemorrhages on the pericardial sac, rib cage epicardium, and abdominal Fats.

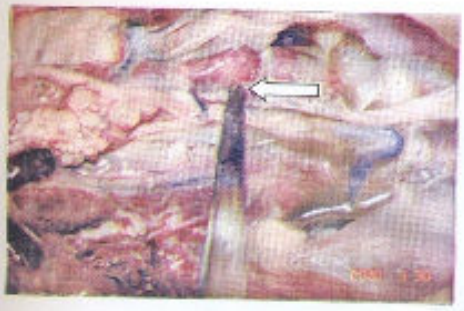


FIG12: Swollen Kidney

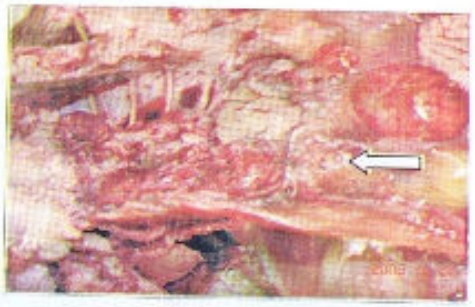


FIG13: Exudates in the peritoneum

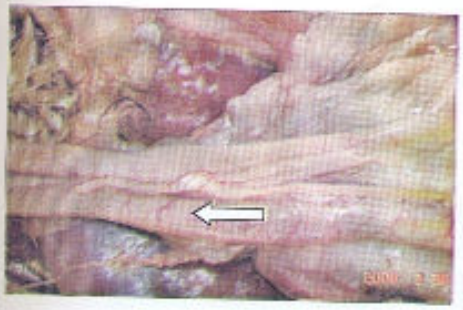


FIG14: Congested duodenal vessels

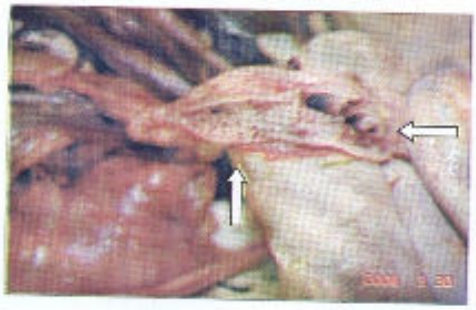


FIG15: Petechine and Ecchymoses of Cecal Tonsil

FIGURE 16: Mucoid intestinal exudates (Jejunum)

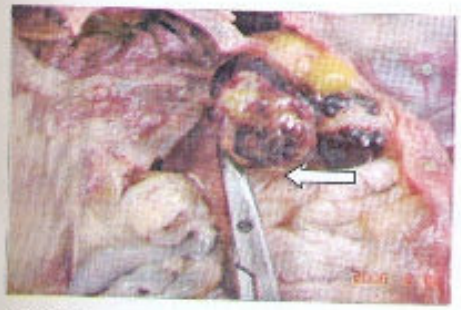


FIG18: Haemorrhagic Ovarian Follicles

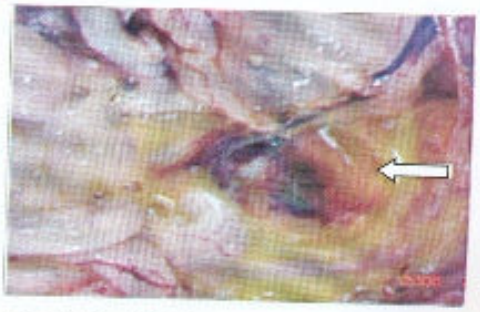
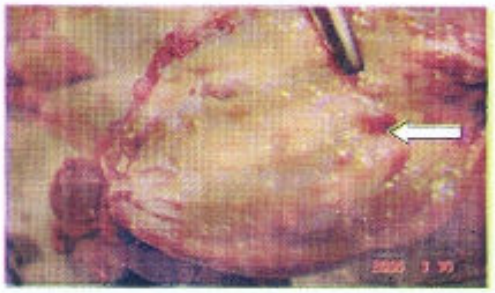


FIG 17: Egg Yolk Peritonitis



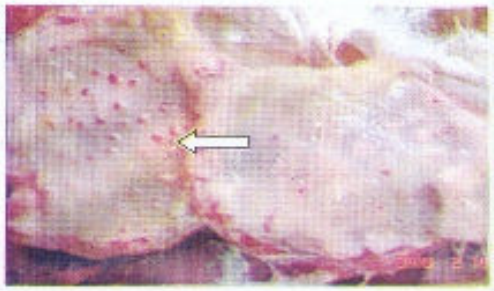


FIG 19: Ecchymoses and Petechiae of the Proventriculus

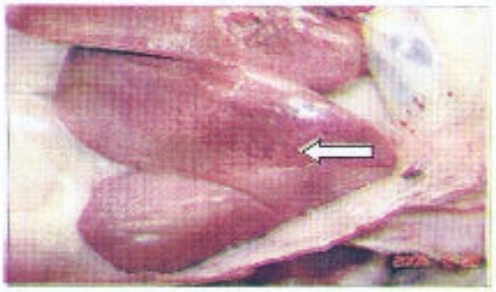


FIG 20: Swollen haemorrhagic liver and serosal fat

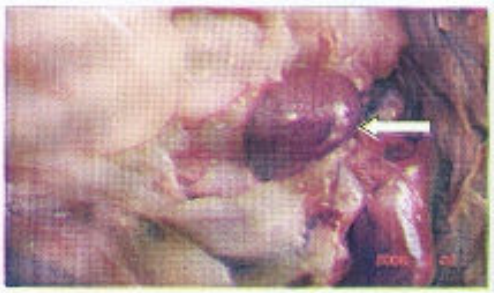


FIG 21: Swollen and congested spleen

DISCUSSION

The presence of swollen heads, eyelids, heads, cervical/neck regions, cyanosis of combs, wattles and featherless areas of the head, subcutaneous haemorrhages, yellowish-green diarrhea, depression, soft shell to shell-less eggs, incordination as well as sudden and high mortalities appear to be the overall most frequently encountered clinical signs. This is in agreement with what is reported elsewhere especially in The Netherlands, Thailand, Japan and Vietnam (2).

In the case of HPAI outbreaks, the gross lesions described in literature are made up of a variety of congestive, haemorrhagic, exudative and necrobiotic changes in both laying hens and turkeys. The internal organs (Spleen, liver, kidney, pancreas and intestine) were enlarged, haemorrhagic and blotched by necrotic foci. Furthermore, pancreatitis, tracheaitis, pneumonia and haemorrhagic caecal tonsils were observed as well as the petechial haemorrhages on the epicardium, abdominal fats and occasionally in the muscles (breast, sternum and thigh).

The gross lesions that were consistently seen in positive cases of HPAI involving layers in this study were congestion/haemorrhages of sinuses, presence of either traheaitis, cloudy air sacs, congestion and exudates in the lungs, peritonitis, petechial haemorrhages affecting the proventriculus, liver, spleen, kidneys myocardium, abdominal adipose tissue and sometimes inflamed bursae. Again, the gross lesions in commercial laying flocks in Nigeria were consistent with what has been reported elsewhere in domesticated commercial flocks (2; 3; 9).

Our study, described the features of AI outbreak caused by H5N1 In outbreaks caused by other HPAI serotypes, the clinical signs and pathological changes may be different. In conclusion, our

study indicates that the clinical signs, gross and microscopic lesions in commercial chickens are similar to those already described in literature.

REFERENCES:

1. Alexander, D. J. (2000): A review of avian influenza. In: *proceedings of the European Society for Veterinary Virologist* (ESVV) symposium on influenza viruses of wild and domestic animals. Ghent. 16-18. May 1999. Veterinary Microbiology, 74, 3-13.

2. Alexander, D.J. (2003). Report on avian influenza in the Eastern Hemisphere during 1997-2002. *Avian Diseases* 47(Special Issue): 792-797.

3. Armin, R. W. E.; Bert, K..; Guus, K., (2004): Performance of gross lesions at post-mortem for the detection of outbreaks during the avian influenza A virus (H7N7) epidemic in The Netherlands in 2003.

4. Hooper, P. and Seller, P., (1998): Pathology of low ands high virulent influenza virus infections. In: D. E. Swayne & R. D. Slemons (Eds), *Proceedings of the 4th International Symposium on Avian influenza*. 29- 31 May 1997, Athens, GA, USA, Pp 134-141.

5. Ilaria Capua and Dennis J. Alexander (2004): Avian influenza: recent developments. *Avian Pathology* 33(4), 393- 404

6. Jacob, J.P; Butcher, G.D; Mather, F. B and Miles, R.D (2003): Avian influenza in poultry, *Institute of food and agricultural sciences extension*, 1-4.

7. OIE/FAO/WHO (2005): Consultation on avian influenza and human health: Risk reduction measures in producing, marketing, and living with animals in Asia, Kaula-Lumpur, Malaysia, July 2005.

8. Trevor M.Ellis, R. Barry Bousfield, Lucy A. Bissett, Kitman C. Dyrting, Geraldine S.M. Luk, S.T. Tsim, Katharine Sturm-Ramirez, Robert G. Webster, Yi Guan and J.S Malik Peiris (2004) Investigation of outbreaks of highly pathogenic H5N1 avian influenza in water fowl and wild birds in Hong Kong in late 2002, *Avian pathology* 33(5) 492-505.

9. WHO (2006). Avian influenza: significance of mutations in the H5N1 virus. 20th February 2006. http://www.who.int/csr/disease/avian_influenza/en.

10. Zanella, A., Dall'Ara, P., and Martino, P. A., (2001): Avian influenza epidemic in Italy due to serovar H7N1. Avian Diseases, 45, 257-261.