

SEROLOGICAL STUDY BY USING THE ELISA TECHNIQUE TO IDENTIFICATION OF AVIAN INFECTIOUS BRONCHITIS DISEASE AMONG SOME FIELDS OF BROILER CHICKENS IN DIYALA PROVINCE.

Amer Alazawy

*Department of Pathology and Avian disease. - College of Veterinary Medicine - University of Diyala

Amer_alazawy@yahoo.com

ABSTRACT

Infectious bronchitis (IB) is a common highly contagious viral disease of poultry; it causes major economic losses to the poultry industry. This study was conducted to investigate the prevalence of avian infectious bronchitis (IB) by evaluated the maternal derived antibodies (MDA) at 3 days of age and compared with the antibodies against the disease in 25 days of age, also to study the clinical signs and gross lesions of the suspected infected birds in some broiler chickens farms of Diyala governorate during the period from September 2012 to April 2013. Twenty broiler chicken flocks were reported in this study, blood samples were collected from each flocks two times to investigate the antibody titers by using Enzyme- Linked Immunosorbent Assay (ELISA). Significant variation ($P<0.05$) was observed in the means of antibody titers against IBV between the maternal derived antibodies at 3 days old and 25 days old, also 14 farms showed clinical signs of IB including mainly, severe respiratory signs with high morbidity and mortality, postmortem examination of affected flocks showed lesions mainly in trachea and kidney. The result of this study showed that IB was the most prevalence and widespread disease in broiler chickens farm particularly at 3 weeks of age. Therefore, because of the lack of a specific vaccine against endemic strains of IBV in Iraq, IB infection has remained a problem in the Diyala poultry farms.

Key words: Infectious bronchitis, ELISA, maternal derived antibodies, Diyala.

INTRODUCTION

Avian infectious bronchitis virus (IBV) is a highly infectious pathogen of domestic fowl that replicates primarily in the respiratory tract but also in epithelial cells of

Received for publication Sept. 10 , 2013 .

Accepted for publication Nov. 10 , 2013 .

the gut, kidney, and oviduct causes a highly contagious respiratory and sometimes urogenital disease of chickens that is characterized by respiratory signs, nephritis, or reduced egg production and quality in layer chickens (Cavanagh and Naqi, 1997). Avian infectious bronchitis (IB) was first described in the United States of America (USA) in the 1930s as an acute respiratory disease mainly of young chickens (Li *et al.*, 2009).

The virus is a member of the genus Coronavirus, family *Coronaviridae*, order Nidovirales. The genome of IBV is a single-stranded, linear positive sense RNA (Cavanagh, 2005; Cavanagh, 2001). IBVs have been identified from all over the world and more may be found in the future, that lead to high morbidity in all ages of chickens and high mortality in chicks less than 3 weeks old (Case *et al.*, 1983). Initially, it was believed all the isolates belong to a single prototype termed Massachusetts (Mass) serotype mostly isolated from commercial poultry (Cavanagh and Naqi, 1997). Subsequently, other serotypes were isolated and it is clear now that a considerable number of different serotypes with antigenic and pathogenic differences exist in different parts of the world (Gough *et al.*, 1992). Since IBV was first described in 1930s, there are more than 30 serotypes of IBV and dozens of virulent strains, with a variation being developed from time to time cause economic loss in chickens. The little or no cross-protection occurs between different serotypes of IBV is well known, therefore, continuous determination of the epidemic serotype and production of new generations of vaccines are crucial for controlling IB in each country (Casais *et al.*, 2003). Vertical transmissions within the embryo have never been reported. But virus may be present on the shell surface of hatching eggs via shedding from the oviduct or alimentary tract (Saif *et al.*, 2008). The main method of protecting poultry from infectious bronchitis (IB) is the administration of both live attenuated and killed vaccines. Because of the lack of a specific vaccine against endemic strains of IBV. IBV infection has remained a problem in the Iraqi poultry industry causing mortality and adverse effects on quantity and quality of egg production as well as renal failure lead to high range of mortality in broiler chickens due to infections with strains serologically different from those used for vaccine serotypes (Lim *et al.*, 2011). The purpose of this study was conducted to investigate the prevalence of avian infectious bronchitis (IB) by the evaluating the (MDA) at 3 days age and compared with the antibodies against the disease in 25 days age also to study the clinical signs and gross lesions on the suspected infected birds in some broiler chickens flocks of Diyala governorate.

MATERIALS AND METHODS

1- Broiler flock

To study the sero-prevalence of IB, blood sample were collected from twenty broiler farms of different selected flocks in different areas of Diyala province during the period from September 2012 to May 2013. Each flock involved

between 5000 to 6000 chicks of Ross 308 breed which were received from different hatcheries, Diyala/Iraq. The chicks were reared for 35 days on floor under almost healthy environmental circumstances. Pelleted feed from a commercial source was provided *ad libitum*. All the flocks had been vaccinated with IB vaccines (mostly H-120) in 3 days age and also vaccinated against Newcastle and Gumboro disease in 12 and 14 days respectively. Commercial vials of live attenuated vaccines (Lyophilized) were used for vaccination program as follow:

- 1- Nobilis IB H120 is a live vaccine against Infectious Bronchitis (Massachusetts H120, 3 Log₁₀ EID₅₀, Intervet – Holland).
- 2- Nobilis ND Clone 30 is a live vaccine against Newcastle disease in chickens (NDV Clone30 strain/Lentogenic).
- 3- Nobilis® Gumboro E228, (A freeze-dried culture of a non-pathogenic strain of Infectious Bursal Disease virus).

2-Serological Examination:

A total of 720 Blood samples were collected from 18 broiler of each flock for the separation of sera to measurement of (MDA) and also sera from chick suspected to be infected with IBV by indirect ELISA on 3rd, days of chick's age (prior to vaccination) and day 25 (Synbiotic Corporation).

Five ml blood was collected aseptically from the wing vein of birds by disposable syringes (Terumo, Japan). Collected blood centrifugation by using (Hettich Centrifuge, Germany) for 10 minutes at 3000 rpm. After collecting the serum, we prepared from detection the (MDA) and also to estimate the suspected infected chickens with infectious bronchitis virus by using indirect ELISA according to the manufacturer's instruction using IBV pre-coated plates and pre-diluted, ready to use reagents and buffer commercial kit(Bio Teck, USA).

3- Symptoms and Gross lesion appearance

Postmortem examination was carried out on the infected chicks and also on the dead carcass. Chickens brought to the poultry diagnosis laboratory, college of veterinary medicine, university of Diyala, by farmers. Five or more chicks per each flock were examined on day 10 and weekly thereafter for 2 weeks, those chickens were in varying ages. All cases from flocks started to show clinical signs such as depression, respiratory distress (rales, sneezing, and gasping), mild serous nasal discharge and an occasional but infrequent gasping reflex accompanied by extension of the head and neck. Some dyspnea with abdominal breathing was observed, more frequently in groups with mixed infections of IBV and avian influenza, sometimes accompanied by lacrimation, facial swelling, and death rate of 40%.

Whereas chickens exhibit renal cases observed mild sneezing and tracheal rales which develop about 24 hours, after this time severe symptoms are evident. The chick stands in a hunched position, eating very little and drinking more than usual, whereas the vent feather are often slightly soiled with watery white droppings with a total mortality of 10% to 15 % . These chickens were necropsied to check different infected organ.

RESULTS AND DISCUSSION

1-Maternal derived antibodies titers:

The measurement of maternal-derived antibodies at 3days of age (as indicated by ELISA test) showed in table 1.

Table 1: Mean titer of maternal derived antibodies (MDA) against IBV at 3 days age with the mean titer of 8331.10.

No of flock	Age (days)	Means of maternal-derived antibody titer against IBV	Coefficient of Variation %CV
1	3days	8185	19.23
2	3days	9310	20.19
3	3days	10193	23.23
4	3days	9864	19.45
5	3days	8720	17.35
6	3days	7649	61.54
7	3days	8749	21.82
8	3days	8300	20.90
9	3days	8178	19.44
10	3days	6574	14.35
11	3 days	9827	20
12	3 days	8906	34
13	3 days	8258	32
14	3 days	5214	30
15	3 days	9864	33
16	3 days	8213	23
17	3 days	6308	22
18	3 days	10527	33
19	3 days	6605	21
20	3 days	8392	30.02

According to the (Table 1) the uniformity of the ELISA titers is an excellent method of determining the (MDA) in a normally distributed flock. If the CV%

is less than 30% the uniformity of the flock was excellent that means the chicks have good immunity from the hens.

2-The measurement of antibodies against IBV virus on 25 days of ages as suspected infected flocks (indicated by ELISA test) showed in Table 3.

Table 2: Mean titer of the flocks after 25 days was 372.2500.

No.	Age (days)	Means antibodies against IB virus on 25 days of ages	CV%
1	25	304	157.01
2	25	279	124.65
3	25	435	140.24
4	25	387	103.12
5	25	228	69.25
6	25	795	69.14
7	25	480	86.55
8	25	459	95.07
9	25	832	99.02
10	25	278	111.05
11	25	1177	114.56
12	25	191	254.85
13	25	149	291.10
14	25	130	133.09
15	25	279	124.65
16	25	173	198.13
17	25	306	198.97
18	25	161	203.89
19	25	228	340.08
20	25	174	193.23

According to the (Table 2) the uniformity of the ELISA titers is an excellent method of determining the suspected infected flocks. If the %CV is greater than 80%, it means that the flock either have a bimodal population or incomplete or non-uniformity of vaccine administration, and the important interpretation here were the flock have early exposure to IB virus.

The measurement of antibodies titers on 25 days age as suspected infected flocks with IBV showed a significant variation ($P < 0.05$) among the MDA at 3 days ages as showed in table 3.

Table 3: Means of antibody titer against IBV virus on 3 days as (MDA) and also on 25 days as the flocks to be suspected infected with IBV. Within a row, the titers of antibodies that do not have common small letter superscripts vary from each other ($P < 0.05$).

Groups	Means ± SE of antibody titer	95% Confidence Interval	
		Lower Bound	Upper Bound
Treatment T1 (MDI at 3 days ages)	8281 ± 233.622 ^a	7808.657	8754.543
Treatment T2 (suspected infected flocks at 25 days ages).	372.250 ± 233.622 ^b	-100.693	845.193

2- Gross lesions determination

Mortality in infected flocks reached between 20-40% during 7-9 days, the noticeable lesions of infected chickens showed lesions that identified primarily in the upper respiratory tract which characterized by severe congestion with serous, catarrhal, or caseous exudates in the trachea, nasal passages, and sinuses (Fig 1).

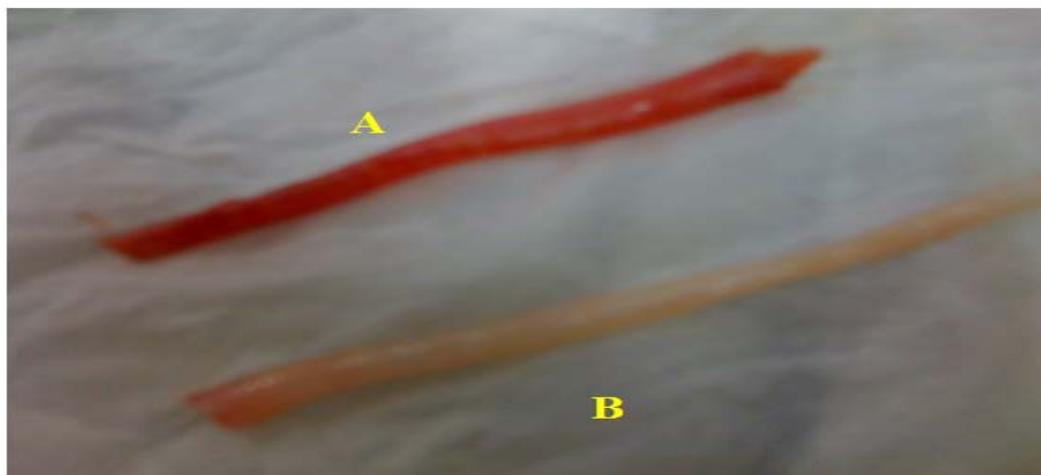


Figure 1, A- Diffuse congestion of tracheal broiler chicken infected with IBV at 3 weeks age
B- Normal trachea of broiler chicken at 3 weeks age

Air sacs may appear mild to severe cloudy or contain yellow caseous exudates that appeared when the infection has been complicated with secondary bacterial infection (Escherichia Coli or mycoplasma) that lead to, airsacculitis, pericarditis and perihepatitis (Fig 2).

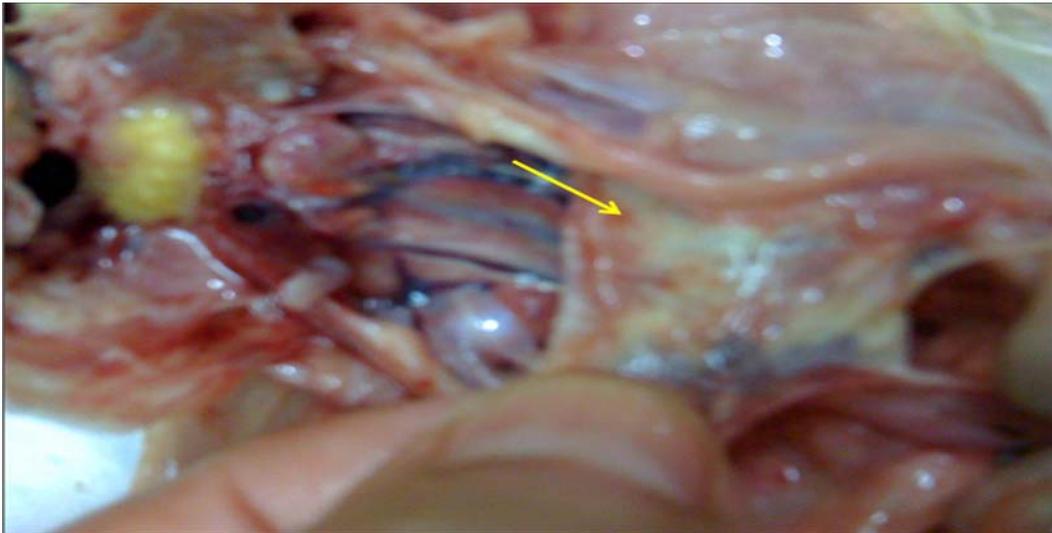


Fig 2. Air sac of infected chickens with IBV shows mild to severe cloudy containing caseous exudates (arrow)

Similar lesions observed with the presence of mycoplasma and E coli that led to more severe clinical signs resulting of pericarditis, perihepatitis and airsacculitis with depressed growth (Fig, 3).

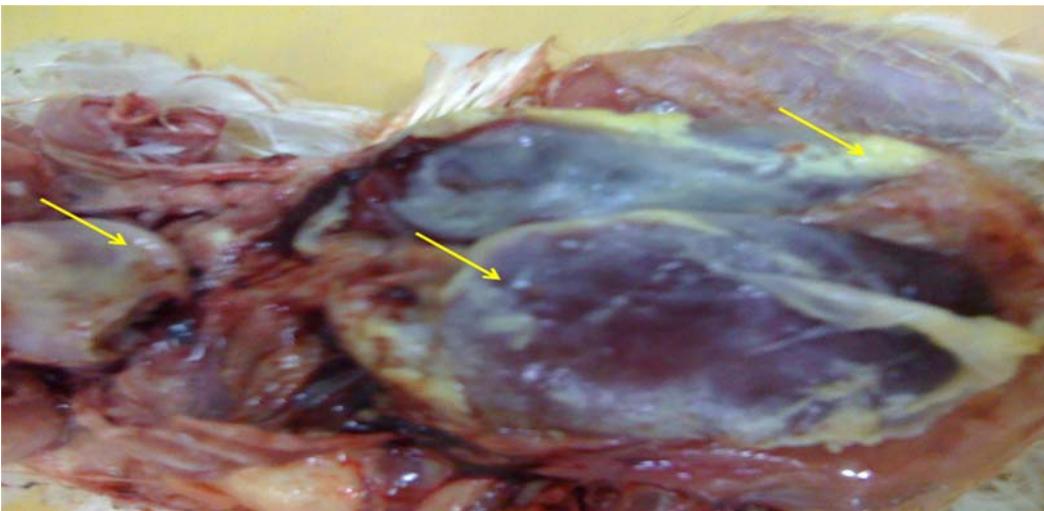


Fig. 3 Infected chick with IBV complicated with secondary bacterial resulting of pericarditis, perihepatitis and airsacculitis (arrow)

In severe infected chickens with IBV, the trachea showed tubular cast formations in the tracheal bifurcation extended into the primary and secondary bronchi were observed in all dead birds in 25 day- old (Fig, 4).

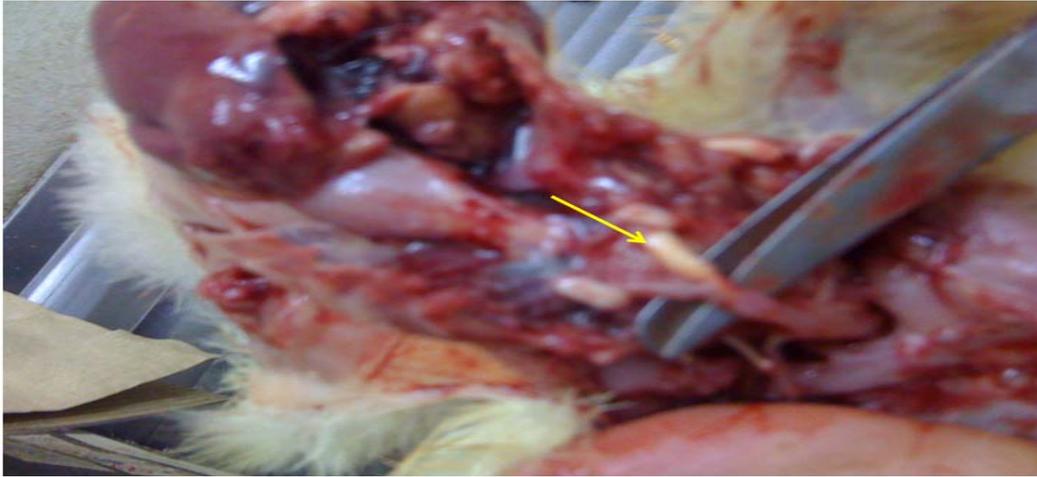


Fig 4, fibrinonecrotic cast formation in the tracheal bifurcation (arrow) at 3 –weeks old broiler chicken infected naturally with IBV

Following infection with nephropathogenic strains, the birds are in good condition demonstrating the rapid course of the disease. The kidneys were pale and were uniformly enlarged from two to four times normal size, with mottled dark and light areas. Focal areas of uric acid precipitation appear in the kidney and may also accumulate in the ureters (Fig, 5).

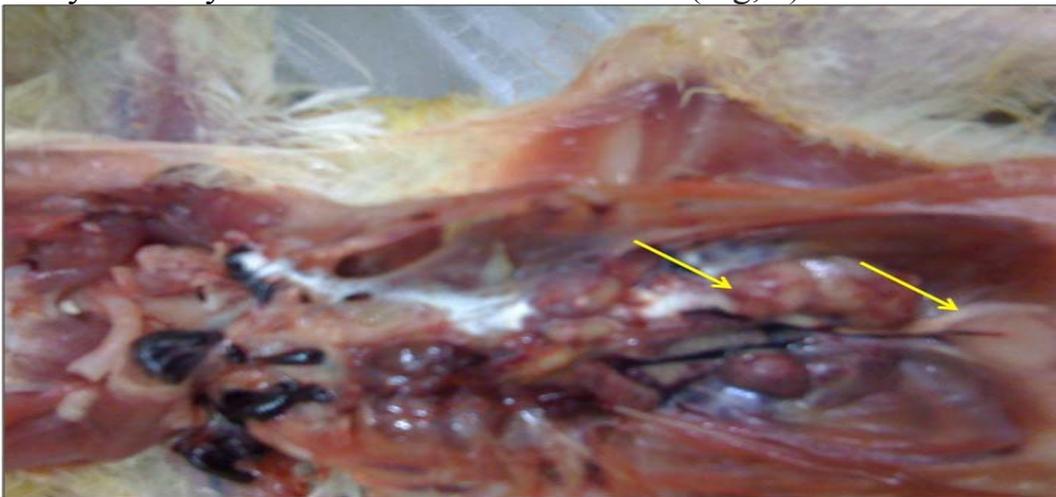


Fig 5- Gross lesion from kidney tissues of broiler chicken infected with IBV showed obvious enlargement and urate deposition in the kidney and in the ureters (arrow).

Infectious bronchitis virus (IBV) is prevalent in all countries with an intensive poultry industry, with the incidence of infection approaching 100%. Vaccination is only partially successful because new serotypes continue to develop due to the continual emergence of antigenic variants (Hopkins, 1978). In recent years a considerable amount of laboratory investigation has been directed at the development of ELISAs for the seroepidemiological detection of exposure to IBV and other viral diseases (Cavanagh, 2007).

Maternal immunity against IB in this study as revealed by ELISA showed high titer (mean titer 8331.10) which appear on table 1. The result of this finding was in agreement with previous published study (Sharma *et al.*, 2000) who mentioned that the development of the reproductive tract of the hens, some of the stimulated lymphocytes localized in the lamina propria of the oviduct and in the stroma of the ovary. Antibody produced locally in these organs usually represented a significant increase of the transferred antibody to the eggs and constantly transfer from the hens to the chick with high titer.

Also this finding was in agreement with previous studies (Luis *et al.*, 1982) which showed that most breeder flocks are periodically vaccinated for both ND and IB, parental antibodies are often transmitted to the progeny at significant levels lead to high titer of maternal antibodies.

Previous study showed that vaccinated flocks against IBV were often applied to reduce economic losses due to an infection with field strains of IBV. Also vaccination procedure, such as using spray vaccination of broiler flocks at 1 day old of age play important role to reduce the viral diseases and induces good clinical protection upon challenge with a virulent IBV strain of the same serotype (Cavanagh and Naqi, 2003; Davelaar *et al.*, 1982). Previous study showed that vaccines against IBV, such as H-120 are often applied to reduce economic losses due to an infection with field strains of IBV (Parsons *et al.*, 1992), whereas in current study, it appeared that, despite the regular vaccination of infectious bronchitis vaccine mostly used H-120 strain, the mean titer were decreased and cannot protected the flocks, and also this finding was in line with (Matthijs *et al.*, 2008) who observed that the majority (67 per cent) of these isolates which causes IBV belonged to serotypes other than the Massachusetts type.

The current study were differ from those (Hofsted and Yoder, 1992)) who mentioned that IBV H-120 vaccine is one of the commonly used live IB vaccines in commercial poultry, and spray vaccination of broilers at 1 day or 15 days of age generally induces good clinical protection upon challenge with a virulent IBV strain of the same serotype.

The present study revealed that the, antibody levels obtained from vaccinated flocks at 25 days of age demonstrated presence of significant differences of the mean titer at 25 days age at level ($P < 0.05$) from MDA at 3 days age, this differences could be due to the flocks vaccinated with H-120 which had the lowest antibody response resulted from the failure of H-120 to induce active immune response in the infected flocks or the flocks showed a very early exposure to disease agent causes by IBV. In contrast to the above findings, the results of current study differ from that of (Luis, 1982) who found that vaccinated the chickens with H-120 led to highest antibody response suggesting a typical response of antibody that make good flocks protection against IBV.

This study also in agreement with the earlier works of (Lue *et al.*, 1993; Arathy *et al.*, 2001) who showed that the reason for outbreaks in vaccinated chickens may be due to the tendency of the virus to undergo continuous genomic drift and shift which led to emerge of new serotypes differences from vaccine strain especially in areas of intensive poultry farming.

The present study showed that, the clinical signs and gross lesions such as respiratory distress (rales, sneezing, gasping and dyspnea) were most sever in bird infected with IBV, also post mortem lesions showed tracheal congestion with serous, catarrhal exudates and cast formation in tracheal bifurcation abruptly reduced airflow, resulting in asphyxiation that was responsible for mortality in dead birds. The findings in the current study are consistent with those of previous works (Jahromi *et al.*,2008; Seifi *et al.*, 2012) who consistently found that the cast formation in tracheal bifurcation were most severe in the AIV and IBV infected chickens which lead to asphyxiation and subsequently responsible for mortality in dead birds.

Whereas previous work of (Tavakkoli *et al.*, 2001; Karimi *et al.*, 2010) observed that infectious bronchitis live vaccine, H-120 strain enhancing the virulence of low pathogenic H9N2 avian influenza virus in broiler chicken flocks which lead to cast formation in tracheal bifurcation.

The current study revealed that the incidence and severity of airsacculitis, pericarditis and perihepatitis may be higher observed in the suspected mycoplasma infection which mixed with IBV in infected flocks and are in agreement with other study of (Chu and Uppal, 1975) who observed that IB infection may precipitate latent mycoplasma infection which showed increased spread and severity of infection.

Whereas previous work of (Kleven *et al.*, 1978) observed that the presence of virus, even the vaccine strain, enhanced the number of mycoplasma isolations and precipitated the incidence of airsacculitis.

The result of present study showed that in some cases, variant strains of IBV seem to be the only agents involved in infectious primary kidney lesions in broiler chickens with mortality of up 25%, which led to focal area of uric acid precipitation appear in the kidneys and may also accumulated in the ureters.

The present finding seem to be consistent with other published studies (Darbyshire *et al.*, 1978) who observed that the initial and principal site of viral replication primarily in the trachea and from this site the virus spreads to other internal organ such as the kidneys with highest titer led to enlarged the kidneys and may be pale or marbled and deposits of urates may be present in the ureters of some chickens.

In conclusion this study obviously demonstrates that the widespread nature of the IBV present in poultry flocks in Diyala province. Future work should included the isolation and serotyping of IBV in any area where this disease is not adequately controlled, so that a suitable vaccination program by using the

common field serotypes as vaccines, can be adopted to protect against IBV caused disease.

REFERENCES

- Cavanagh, D., S and Naqi. 1997. Infectious bronchitis, p. 511–526. In B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoda (ed.), Diseases of poultry, 10th ed. Iowa State University Press, Ames, Iowa.
- Li, L., C. Xue., F.Chen., J. Qin., Q. Xie and Y. Bi. (2009). Isolation and genetic analysis revealed no predominant new strains of avian infectious bronchitis virus circulating in South China during 2004–2008. *Vet Microbiol.* 2010; 143:145–54.
- Cavanagh, D. 2003. Severe acute respiratory syndrome vaccine development: experiences of vaccination against avian infectious bronchitis coronavirus. *Avian Pathol.*, **32**, 567–582.
- Cavanagh, D. 2001. A nomenclature for avian coronavirus isolates and the question of species status. *Avian Pathol.* 30:109–115.
- Case, J.T., A.A.Ardans., D.C.Bolton and B.J.Reynolds.1983. Optimization of parameters for detecting antibodies against infectious bronchitis virus using an enzyme-linked immunosorbent assay: temporal response to vaccination and challenge with live virus. *Avian Diseases* 27:196-210.
- Gough, R.E., M.C. Randall., D. J. Dagless., W. J. Cox, and D. Pearson. 1992. A “new” strain of infectious bronchitis virus infecting domestic fowl in Great Britain. *Vet. Rec.*, 130: 493–494.
- Casais, R., B. Dove., D. Cavanagh, and P. Britton.2003. Recombinant avian infectious bronchitis virus expressing a heterologous spike gene demonstrates that the spike protein is a determinant of cell tropism. *J. Virol.*, 77: 9084-9089.
- Saif, Y.M., A. M. Fadly., J. R.Glisson., L.R. McDougald., L.K. Nolan and D.E.Swayne. 2008. Diseases of poultry, 12th edn. Iowa State University Press, Ames, Iowa, pp 117–130.
- Lim, T.H., H. J.Le., D. H. Lee., Y. N. Lee., J. K.Park and H. N. Youn. 2011. An emerging recombinant cluster of nephropathogenic strains of avian infectious bronchitis virus in Korea. *Infect Genet Evol.* 11:678– 85.
- ELISA – SYNBIOTICS Corporation. (1997-2003). www. Symbiotic.com.
- Hopkins, S.R .1978. Typing field isolates of infectious bronchitis by the plaque-reduction test. *Avian Dis.* 22:71-81.
- Cavanagh, D.2007. Coronavirus avian infectious bronchitis virus. EDP Sciences., *Vet. Res.*, 38: 281-297.
- Sharma, J.M., I. J. Kim., S. Rautenschlein and H. Y.Yeh.2000. Infectious bursal disease virus of chicken: pathogenesis and immunosuppression. *Develop. Comp. Immunol.*, 24: 223-235.

- Luis, F., A. P.Andrade and O.J. Fletcher.1982. Vaccination of Day-Old Broilers against Infectious Bronchitis: Effect of Vaccine Strain and Route of Administration.*Avian Dis.*, 27,178-187.
- Cavanagh, D and S. A. Naqi. 2003. Infectious bronchitis. In: Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE (eds). Diseases of Poultry, 11th Ed. Pp: 101-119. Ames, Iowa States University Press, USA.
- Davelaar, F.G., B. Kouwenhoven and A. G.Burger.1984. Occurrence and significance of infectious bronchitis variant strains in egg- and broiler production in the Netherlands. *Vet. Q.*, 6: 114–120.
- Parsons, D., M.M.Ellis.,D. Cavanagh and j. k.Cook.1992. Characterisation of an infectious bronchitis virus isolated from vaccinated broiler breeder flocks. *Vet. Rec.* 31;131(18):408-11.
- Matthijs, M. G. R., A. Bouma., F. C. Velkers., J. H. H. van Eck and J. A. Stegeman .2008. Transmissibility of Infectious Bronchitis Virus H120 Vaccine Strain among Broilers under Experimental Conditions. *Avian*: 3, 461-466.
- Hofstad, M.S. and H. W. Yoder. 1996. Avian infectious bronchitis-virus distribution in tissues of chicks. *Avian Dis.*, 10: 230-239.
- Lu, Y. S., H. W. Shieh., H. J. Tsai., D. E. Lin. and Y. L. Lee. 1993. The incidence and virus isolation of infectious bronchitis in chickens in Taiwan. *J. Chinese Soc. Vet. Sci.* 19:119-129.
- Arathy, S. A., S .Gopalakr., S.M. Kumthekar., D. Thomas and R.N. Sharma. 2011. Seroprevalence of Infectious Bronchitis Virus in Birds of Grenada. *Inter. J. Poult. Sci.*,10 (4): 266-268.
- Jahromi, M., K. Asasi, K., Nili, H., Dadras, H and H. Shooshtari. 2008. Coinfection of avian influenza virus (H9N2 subtype) with infectious bronchitis live vaccine. *Arch. Virol.*, 153: 651-655.
- Seifi, S., K. Asasi and A. Mohammadi. 2012. An experimental study on broiler chicken co-infected with the specimens containing avian influenza (H9 subtype) and infectious bronchitis (4/91 strain) viruses. *Iranian J. Vet. Res.*, 39: 138-142.
- Tavakkoli, H., K. Asasi., A. Mohammadi. 2011. Effectiveness of two H9N2 low pathogenic avian influenza conventional inactivated oil emulsion vaccine on H9N2 viral replication and shedding in broiler chickens. *Iranian J. Vet. Res.*, 12: 214-221.
- Karimi, M.,M. Ansari., K. Asasi and H.Nili .2010. Risk factors for detection of bronchial casts, most frequently seen in endemic H9N2 avian influenza infection, in poultry flocks. *Prev. Vet. Med.*, 95: 275-280.
- Chu, H.P and P. K.Uppal .1975. Single and mixed infections of avian infectious bronchitis virus and Mycoplasma gallisepticum. *Dev Biol Stand* : 28:101-114.

- Kleven, S.H., C. S.Eidson and O.J.Fletcher. 1978. Airsacculitis induced in broilers with a combination of *Mycoplasma gaO.Jllinarum* and respiratory viruses .*Avian Diseases*, 22, 707-716.
- Darbyshire J.H., J.K. Cook and R.W. Peters R.W.1978. Growth comparisons of avian infectious bronchitis vims strains in organ cultures of chicken tissues. *Arch. Virol*, 56, 317-325.

دراسة سيروولوجية باستخدام تقنية الاليزا للتحري عن مرض التهاب القصبات الخمجي بين مجموعة من حقول الدواجن في محافظة ديالى .

عامر خزعل صالح العزاوي

*فرع الأمراض وأمراض الدواجن - كلية الطب البيطري- جامعة ديالى .

المستخلص

فايروس التهاب القصبات الهوائية الخمجي من الامراض الشائعة الحدوث والشديدة العدوى والذي يسبب خسائر اقتصادية فادحة في مجال تربية الدواجن وهو شائع الحدوث في جميع البلدان التي تربي فيها الدواجن. صصمت هذه الدراسة لمعرفة مدى شيوع مرض الالتهاب القصبات الهوائية الخمجي في حقول تربية دجاج اللحم في محافظة ديالى من خلال دراسة سيروولوجية باستخدام تقنية الاليزا. اجريت الدراسة في مختبر الاحياء المجهرية – كلية الطب البيطري /جامعة ديالى. تم حساب المناعة الاموية للافراخ بعمر 3 أيام وكذلك تم حسابها بعمر 25 يوماً لمعرفة التفاوت في هبوط مستوى الاجسام المناعية استنادا الى التعليمات المرفقة مع العدة التشخيصية الخاصة بهذا المرض والمصنع من شركة سمبايوتك الامريكية حيث دل الانخفاض في مستوى الاجسام المناعية ($P < 0.05$) بعمر 25 يوماً إلى تعرض القطيع للاصابة المبكرة من خلال الهبوط في مستوى الاجسام المناعية وكذلك تمت دراسة الأعراض السريرية والمتمثلة بصعوبة التنفس والسعال والغرغرة وخروج مواد مخاطية من المنخرين وكذلك دراسة التغيرات المرضية المرافقة للحالات المصابة من خلال دراسة الصفات التشريحية لكل حالة والتي اظهرت وجود مواد سائلة ومتجينة في القصبة الهوائية وكذلك تغيرات في الاكياس الهوائية والكلى. اظهرت نتائج الدراسة شيوع هذا المرض في حقول الدواجن في محافظة ديالى وخاصة في عمر 3 اسابيع وعدم فاعلية اللقاحات المستخدمة ضد المرض.

الكلمات المفتاحية: التهاب القصبات الخمجي، الاليزا ، المناعة الاموية ، ديالى.