Serological and Histopathological Studies on Infectious Bronchitis in Non Vaccinated Layers and Broiler Chickens in Al- Jabel El-Khder in Libya

Nawara M. Eissa¹, Ajlal A. Alzergy*², Azza El- Sawah³, Mahmood Ezzat⁴ and Fauzia Elgany⁵

¹Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine Omar Al Mukhtar University, Al Bayda Libya.
²*Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Omar Al Mukhtar University, Al Bayda Libya.
³Department of Poultry Diseases, Faculty of Veterinary Medicine Omar Al Mukhtar University, Al Bayda Libya.
⁴Department of Microbiology, Faculty of Veterinary Medicine Omar Al Mukhtar University, Al Bayda Libya
⁵Central Veterinary laboratory at Technical Center of Animal Health and Animal Breeder

Corresponding Author: Nawara M. Eissa

Abstract: This study was aimed to characterize the sero-prevalence and histopathological lesions on infectious bronchitis (IB) in non vaccinated layers and broiler chickens in Al Jabel El-Khder - Libya using Agar Gel Immunodiffusion (AGID) and ELISA test. A total of 150 serum samples were collected from non vaccinated layers and broiler chickens for serological IB investigation. IB antibodies were detected in serum samples using AGID test and ELISA test. All tested farms were ELISA positive for IB antibodies, the percent of positivity were 42% and 75% in samples from broiler and layer chicken respectively. Severe clinical signs and gross lesions in infected chickens included drop in egg production, decline of egg quantity and quality, as well as loss of body weight and mortality reached 30% among flock of layers chicken with a total population of 40200 birds in a large poultry farms at Al-weseta region in Al Jabel El-Khder - Libya were recorded. Also, respiratory manifestation in broiler birds of 21 day old with mortality rate 30% and loss of body weight in broiler chicken farm with a total population of 6000 birds were observed. Post mortem examination of illness birds and freshly dead birds revealed sever renal congestion, shorting and narrowing of oviduct, congestion in trachea, airs aculitis, caseous plugs in trachea and two bronchi and peritonitis. Tissue samples (lung, trachea, spleen, ovary, oviduct, kidney, liver, cerebellum and spinal cord) were collected and processed for histological studies by light microscopy. Examination of histological sections of trachea, lung, kidney ovary, oviduct, kidney, liver, cerebellum and spinal cord of non vaccinated naturally infectious bronchitis virus (IBV) infected chicken revealed many and sever histopathological alterations in all tested tissue which were more pronounced in oviduct and kidney.

Key Words: Serology, histopathology, infectious bronchitis virus, non vaccinated laying and broiler chicken and Agar Gel Immunodiffusion and ELISA test

Date of Submission: 04-03-2019

I. Introduction

Infectious bronchitis is an acute, highly contagious, and primarily respiratory infection in chickens, occurring at all ages. Infectious bronchitis virus (IBV) is present in most poultry producing areas [1]. Infectious bronchitis virus has a great economic effect on the layer industry, because it affects egg production. Besides respiratory lesions, early exposure to IBV causes extensive damage to a wide range of epithelial surfaces in various parts of the body [2]. Infectious bronchitis (IB) is one of the major economically important poultry diseases distributed worldwide. It is caused by infectious bronchitis virus (IBV) and affects both galliform and nongalliform birds. Infectious bronchitis (IB) is an acute, highly contagious, and primarily respiratory infection in chickens, occurring at all ages. Its economic impact includes decreased egg production and poor egg quality in layers, stunted growth, poor carcass weight, and mortality in broiler chickens [3]. IBV is caused by a corona virus, of the family Corona viridae with over twenty five known genotypes worldwide [4]. It is a single stranded RNA, envelope virus IBV is present in most poultry producing areas [1]. The disease was first identified in North Dakota, USA, when Schalk and Hawn reported a new respiratory disease in young chickens [5]. Since then, IBV has been recognized widely, especially in countries with large commercial poultry populations. IBV has a wide geographical distribution and it was found in regions of Africa, Asia, Australia, Europe, and the
America's [6] [7]. IBV was first detected in Libya in 2012, a part of which have a strong relationship with the Egyptian strains [8]. IBV was initially found to cause only respiratory disease but over time the virus has been isolated from various non-respiratory tissues including kidneys and different parts of the oviduct and alimentary tract [9] [10]. In IBV infected flocks, the morbidity rate can reach 100% but the mortality rate depends on the presence of secondary infections, flock age, immune status, management and environmental factors. Even though all age group of chicks are susceptible to IBV, baby chicks are more susceptible than older ones [11]. The typical signs of IBV in chickens of less than six weeks of age are depression, huddling under the heat source, difficulty with breathing (often more noticed at night when the birds are resting), gasping, coughing, nasal discharge, lethargy, watery eyes and mildly swollen sinuses in severe conditions [11] [12]. Infection of reproductive tract is associated with lesions of the oviduct, leading to decreased egg production and quality. Eggs may appear misshapen, rough-shelled, or soft with watery egg yolk [3]. Histological findings include interstitial nephritis, tubular degeneration, and infiltration by heterophils [3]. Granulocytic and urate casts in the collecting duct and occasional necrotic foci and lymphocytic infiltration in the interstitial space were persistent throughout the infected birds. Also, moderate edema in Bowman’s capsule and loss of cilia until epithelium lining oviduct and lymphocytic and plasma cell infiltration in the subepithelial space was persistent in bled infected with 2 different strains of infectious bronchitis virus (T and N1/88) [13] [14]. Large accumulation of yolk fluid may be seen in the abdominal cavity, Cystic oviduct has also been observed in young layers following infection with certain IBV strains [3]. ELISA assays are more sensitive and easily applied for field use and in monitoring antibody response following vaccination or exposure. However, emergence of different IBV serotypes that do not cross-react with commonly available anti-sera generally made serological tests less applicable and non-conclusive in classifying new or emerging IBV isolates [15] [16].

II. Materials and Methods

Prevalence of infectious bronchitis virus (IBV) was studied in commercial poultry farms. A total population of 40200 birds and there was respiratory manifestation in broiler chicken farm, 21 day old, the total number 6000, with mortality rate (30%) and loss of body weight. These farms had history of mortality at early age, clinical signs like respiratory, renal and reproductive dysfunction. Dead birds (carcasses) were collected in sterilized polythene bags, correctly labeled and brought to laboratory and kept in refrigerator at 4°C until further used. On Post-mortem examination, gross pathological lesions (serous exudates and haemorrhage in trachea, air sacculitis, visceral and articulate gout) were noted. The tissue samples of lungs, trachea, kidney and cloacal swab were collected. The blood samples were collected from 150 flock of layers chicken in a large poultry farms at Al-weseta in Jabel El-Akhdar.

Chicken sera:

Field sera: 150 blood samples were collected from suspected cases of broiler and layers chicken showing respiratory signs, poor weight gain in broilers, as well as decreased both egg production and egg quality in layer during January 2012. The serum were kept at -20°C till serologically examined for IBV antibodies, according to Anon [17].

Agar Gel Immunodiffusion (AGID) Test

This technique was based on the ability of antibodies to form precipitation lines specifically with the antigen. The free diffusion of the antigen and the antibody takes place in an agarose gel, which creates lines of precipitation visible to the naked eye. The procedure was followed according to Ouchterlonry [18] with some modifications

ELISA test

Buffers and Solutions: phosphate buffered saline, pH 7.2, carbonate-bicarbonate coating buffer, wash and dilution buffer, and blocking buffer were prepared according to Kok et al [19]

Antigen preparation: The IBV vaccine was used directly as described by Abdel-Moneam [20] for the coating of ELISA plates. The optimal dilution of antigen, serum and peroxidase conjugate for indirect ELISA was performed by control panel titration [21]

ELISA test proper: The methods of De Wit et al. [22] were used to determine serum positive standard antibody titers, various positive antisera, and negative sera. Sample / positive ratio method (S/P ratio):

Snyder and Marquardt [23] were determined and the standard curve was constructed.

II-Histopathological studies

For the light microscopic examination the samples were immediately taken and cut into pieces of desired size and fixed in aqueous Bouin’s fixative (Saturated picric acid 75 ml + conc. formalin 25ml + glacial acetic acid 5 ml) for 24 hours, washed in running tap water for remove extra Bouin’s fixative, dehydrated in
ascending grades of ethyl alcohol, cleared in xylene, impregnated in paraffin wax (melting point between 56°C and 58°C), 5 μm thick histological sections were cut and stained with Harri’s hematoxylin and eosin (H&E) according to Bancroft and Gamble [24]. Stained sections were examined under light microscope and histopathological changes were recognized and photographed using light microscope equipped with camera head (Nikon Eclipse E400, Japan).

III. Results

Seroprevalence of IBV ELISA antibody:
A total of 150 serum samples were collected from non vaccinated layers and broiler chickens for serological IB investigation IB antibodies were detected in serum samples using AGID test and ELISA test. the result proved that all flocks were sero-positive for IB antibodies, the percent of positivity were 42% and 75% in samples from broiler and layers chicken respectively.

Clinical findings, Gross necropsy findings:
Many clinical signs and gross lesions in non vaccinated naturally IBV infected chickens were observed. Such symptoms include drop in egg production (25%), decline of egg quantity and quality (rough shell, shell less eggs and watery albumin), respiratory manifestation (sneezing, coughing and rales), decrease of feed intake and conversion, loss of body weight and poor growth in broilers. Beside neurological symptoms and neck twisting post mortem examination of illness birds and freshly dead birds revealed sever pale and swollen kidneys, renal congestion, shorting and narrowing of oviduct, congestion in trachea, airs aculitis, caseous plugs in trachea and two bronchi and peritonitis. congestion of tracheal mucosa and extrapulmonary bronchi. As well as high rate of mortality reached 30% among flock of layers chicken in a large poultry farms at Al-weseta region in Al Jabel El-Khedar - Libya with a total population of 40200 birds was detected. Also, respiratory manifestation and high rate of mortality (30%) in broiler birds of 21 day old in broiler chicken farm with a total population of 6000 were recorded.

Histopathological findings:
Histopathological examination of the Lung of non vaccinated naturally IBV infected chickens showed destrected and thick alveolar wall accompanied by oedema, many infiltration cells aggregated around and intrabrounchioles (Figs.1 and 2). The most prominent histopathological changes in the trachea of naturally IBV infected chickens were hyperplasia, lymphycytic infiltration lymphocytes into the submucosa and oedema, diffuse lymphycytic aggregation, degeneration of the epithelium mucus, and haemorrhages, moderate to severe inflammatory cell infiltration of upper respiratory tract mucosa, resulting in thickened and more compact mucosa (Figs.3 and 5). Histological examinations of Ovary of non vaccinated naturally IBV infected chickens showed extensive haemomoraye and many destrected cells with central chromatolysis, decrease and irregular follicles (Figs. 6 and 7). Oviduct sections of non vaccinated naturally IBV infected chickens appeared highly destructed with massive lymphocyte infiltration (Figs. 8 and 9). Histopathological changes include massive renal haemorrhages and degeneration renal tubular epithelium, focal lymphocytic infiltration aggregation in the interstitium and in the glomeruli, as well as degenerative changes in tubular epithelium, urates deposition and degenerative changes in renal tubules, oedema, rounding and sloughing of epithelial cells, and focal lymphocytic infiltration were observed in the kidney of naturally IBV infected chickens. Also, necrosis of the proximal convoluted tubule with loss of cilia of lining epithelium, distension of distal convoluted tubule, necrotic foci, heterophils, and lymphocytes in the interstitial spaces and oedsured Bowman’s capsule were detected (Figs.10 and 11). The liver of non vaccinated naturally IBV infected chickens showed marked lesions included vacuolated hepatocytes, Proliferation of Kupffer cells and focal necrotic areas associated with severe mononuclear leukocyte inflammatory infiltration (Figs.12 and 13). Also, many lesions in form decrease number of purkinje with abnormal feature and decrease cells population in granular layer in cerebellum cortex (Fig.14) beside many giant multipolar neurons in grey matter of spinal cord of non vaccinated naturally IBV infected chicken were seen (Fig.15). Moderate depletion of cellularity in red and white pulp beside reduce splenic corpuscles were observed in the spleen of non vaccinated naturally IBV infected chicken (Fig.16).
Figs.(1&2): Lung of non vaccinated naturally IBV infected chicken showing destructed and thick alveolar wall accompanied by oedema, many infiltration cells aggregated around and intrabrounchioles (H&E stain, 400X). Fig.(3-5): Trachea of non vaccinated naturally IBV infected chicken showing Hyperplasia of the epithelial layer cells destructed epithelium and infiltration of lymphocytes and heterophils desquamation of epithelial cells destructed epithelium and a dense lymphocytic infiltration in the tracheal mucosa (H&E stain, 400X).

Figs.(6&7): Ovary of non vaccinated naturally IBV infected chicken showing extensive haemorrage and many destructed cells with central chromatolysis, decrease and irregular follicles (H&E stain, 400X). Figs.(8 and 9): Oviduct of naturally IBV infected chickens showing highly destructed with massive lymphocyte infiltration (H&E stain, 400X).
**Fig.(10 and 11):** Kidney of non vaccinated naturally IBV infected chicken showing acute nephritis with congestion and necrosis of urinary tubules, massive renal haemorrhages, oedema, and degeneration renal tubular epithelium with loss of cilia, rounding and sloughing of epithelial cells, desquamation of epithelial cells, infiltration of lymphocytes and heterophils and obscured urinary space (H&E stain, 400X). **Figs.(12 and 13):** Liver of non vaccinated naturally IBV infected chicken showing marked vaculated hepatocytes, Proliferation of Kupffer cells and multifocal necrotic area with severe mononuclear leukocyte inflammatory infiltration (H&E stain, 400X).

**Fig.(14):** Cerebellum cortex of non vaccinated naturally IBV infected chicken showing decrease Purkinje with abnormal feature, decrease aggregation cells in granular (H&E stain, 400X). **Fig.(15):** Spinal cord of non vaccinated naturally IBV infected chicken showing giant multipolar neuron in grey matter (H&E stain, 400X). **Fig.(16):** Moderate depletion of cellularity in red and white pulp beside reduce splenic corpuscles in the spleen of non vaccinated naturally IBV infected chicken (H&E stain, 400X).

**IV. Discussion**

Infectious bronchitis (IB) is an acute, highly contagious and economically important viral disease that occurs in commercial chickens of all ages [25]. Although, the infectious bronchitis virus (IBV) first was believed to occur primarily in young chickens, however, chickens of all age are also susceptible [26]. Infectious bronchitis is an acute highly contagious viral disease of respiratory and urogenital system of chickens affecting
both broilers and layers. Avian infectious bronchitis still poses a great challenge for the chicken industry worldwide [8]. Infectious bronchitis (IB) causes significant economic losses to the poultry industry worldwide. IBV is a highly infectious pathogen and the infected birds usually develop clinical signs very rapidly, within 36-48 hours [26] [27]. The ELISA is considered a useful tool for routine laboratory diagnosis of IBV antigen. In the present study, IB sero-prevalence has been reported on the basis of ELISA and AGID for diagnosis of IBV and serum samples were collect from clinically affected farms with symptoms of coughing, sneezing, tracheal coarse crackles, nasal discharge, decrease of feed intake and conversion, loss of body weight and poor growth in broilers. All tested farms were ELISA positive for IB antibodies, the percent of positivity were 42% and 75% in samples from broiler and layer chicken respectively, while the prevalence rate of disease was also reported through ELISA by many author [28] [29] [30] [31] as 85.3 per cent in Iran, 100 per cent in Gajipur of Bangladesh, 82.7 per cent in southwestern Nigeria, 93.48 per cent in central and western plateau of India respectively. In North Africa, IBV have been reported in Egypt, Tunisia, Morocco and Libya [20][32].

Infectious Bronchitis Virus (IBV) was initially found to cause only respiratory disease but over time the virus has been isolated from various non-respiratory tissues including kidneys and different parts of the oviduct and alimentary tract[9][10][34][35]. In the current work many clinical signs and gross lesions include drop in egg production, decline of egg quantity and quality, respiratory manifestation (sneezing, coughing and rales), decrease of feed intake and, loss of body weight and poor growth in broilers birds. Beside neurological symptoms and neck twisting were observed in non vaccinated naturally IBV infected chickens. Also, severe pale and swollen kidneys, renal congestion, shorting and narrowing of oviduct, congestion in trachea, airs aculitis, caseous plugs in trachea and two bronchi and peritonitis, congestion of tracheal mucosa and extrapulmonary bronchi were seen. In addition, high rate of mortality (30%) among flock of layer and broiler (21 day old) chicken in a large poultry farms at Al-weseta region in Al Jabel El-Khdar-Libya was recorded. In this study, the clinical signs and reduced body weight, are in agreement with previous reports [25][35][36][37]. Kinde [38] and Mahgoub et al. [39] were also recorded similar clinical findings. In uncomplicated cases, food consumption and weight gain were significantly reduced within 3 days after infection and the chickens became depressed [40]. Infection at an early age may cause permanent damage to the reproductive tract leading to a decrease in quantity and quality of eggs[12] [35][41]. Also, Cook and Mockett [42] stated that infection in laying birds results in decreases in egg production and the extent of the damage can vary depending on the time of the infection and hens health status.

Infectious Bronchitis Virus is responsible for causing respiratory, renal and urogenital disease. Such disease is characterized by high mortality rates in affected flocks and severe economic losses due to production loss by reduction in weight gain by broilers and drop in egg quality and productivity in laying poultry birds [26][43][44][45]. Infectious Bronchitis Virus (IBV) is known to affect multiple systems of the host involving lungs, oviduct and kidneys [6][7][11]. IBV infected birds usually develop clinical signs very rapidly, within 36-48 hours. The virus replicates primarily in the upper respiratory tract, leading to viraemia, and then spreads to other organs [41]. Usually, the virus is present in high concentrations in the upper respiratory tract during the first 3-5 days post infection [46][47]. This disease is characterised primarily by respiratory signs, but some IBV strains may also infect other organs such as the intestinal and urogenital tract [25]. Infectious bronchitis virus usually causes reproductive disorders, with a decline in egg production accompanied by soft-shelled and misshapen eggs, inferior shell quality, and thin, watery albumen [48]. Nephropathogenic strains, in addition to tracheal lesions, also induce prominent kidney lesions with mortality of up to 25% in broilers [48]. The same author also reported that nephropathogenic IBV strain causes high mortality, compared with strains infecting only the respiratory or reproductive systems. IB infections may lead to mortality up to 20-30% or higher at five to six weeks of age in chicken flocks [49][50]. In IBV infected flocks, the morbidity rate can reach 100% but the mortality rate depends on the presence of secondary infections, flock age, immune status, management and environmental factors. In young chickens, the mortality rate is typically 25-30% but it can approach 80% depending on the virulence of the strain. Even though all age group of chicks are susceptible to IBV, baby chicks are more susceptible than older ones [11]. Infectious bronchitis virus has a great economic effect on the layer industry, because it affects egg production. Besides respiratory lesions, early exposure to IBV causes extensive damage to a wide range of epithelial surfaces in various parts of the body [2]. However, intrinsic factors such as age could also influence the pathogenesis of IBV effects on the oviduct [51]. Avian IBV is a highly contagious pathogen of chickens that replicates primarily in the respiratory tract and also in some epithelial cells of the gut, kidney and oviduct [52]. The most notable symptoms are those which affect the respiratory tract, hence the term infectious bronchitis. The severities of the clinical signs are influenced by several factors associated with the field virus (such as strain, virulence and dosage), host (age, sex, type and immune status), environment (cold, heat, dust, ammonia and stress) and management and biosecurity levels [12]. Bande et al. [3] reported that morbidity due to IBV infection can reach up to 100%. Clinical signs include depression, wet droppings, and excessive water intake. Infection of reproductive tract is associated with lesions of the oviduct, leading to decreased egg production and quality. Eggs may appear misshapen, rough-
Serological and Histopathological Studies on Infectious Bronchitis in Non Vaccinated Layers a....

shelled, or soft with watery egg yolk [3]. Renal damage associated with different IBV strains is an increasingly important feature of the infection, especially in broilers, as nephropathogenic strains produce less respiratory signs [53] and lesions [54], but can induce high mortality [22]. The mortality ranged from 5.4 to 35.2% with average mortality of 12.2% were recorded. Hofstad [55] recorded mortality up to 25 percent in young chick and Gaba et al. [56] observed mortality up to 30% which are in agreement to the findings of the present study. The necropsy revealed serous exudates and haemorrhage in trachea. Visceral and articular gout with swollen, pale and inflamed kidneys indicated the involvement of nephropathogenic avian IBV. Similar findings were reported by Ni et al. [57]. In younger chick affected with IB, nephritis, mortality up to 25% is common [58][59]. IB is clinically characterized by respiratory rales, coughing and sneezing [60]. Some IBV strains replicate in gastrointestinal tract, oviduct and kidneys due to their nephropathogenic properties, they have potential to cause up to 44% mortality [43]. Some strains of the virus cause severe kidney damage and may be associated with high mortality [61]. Generally, nephropathogenic IBV strain causes high mortality, compared with strains infecting only the respiratory or reproductive [49].

Histopathological examination of non vaccinated naturally IBV infected chickens revealed histopathological lesion in many organs. The histopathological results of the present work were consistent with previous reports about this serotype of IBV [35][36][62][63]. Also, Gola et al. [64] who study histopathological changes in field cases of infectious bronchitis in chickens reported that the trachea of affected birds on histopathological examination revealed deciliation, desquamation of epithelial cells and serous exudation. Trachea of some birds showed haemorrhage. Lungs showed congestion, severe bronchopneumonia and infiltration of inflammatory cells. Kidney revealed tubular necrosis, hyaline degeneration, desquamation of epithelial cells, congestion, infiltration of inflammatory cell and deposition of urate crystal crystal surrounded by inflammatory cell. Microscopic examination of liver revealed mild to moderate degeneration and necrosis of , congestion, infiltrate ion of inflammatory cells and dilatation of sinusoidal space and distortion of hepatic cord [64]. Nephropathogenic IBV strains cause nephritis characterized by swelling and congestion of the kidney , sometimes with pallor of ureters that contain urate deposits [3]. Similarly, infection with nephropathogenic IBV strains may result in pale, swollen, and mottled kidneys [65][66]. Histological findings include interstitial nephritis, tubular degeneration, and infiltration by heterophils [3]. In some cases, necrotic and dilated tubules are filled with urates and casts [11]. Nephropathogenic IBV strains are most described in broiler-type chickens [3]. The other forms of the disease are mainly associated with the reproductive, renal and gastrointestinal systems. It has been reported that the virus can cause infertility in male chickens [67], and the virus has been isolated from the testicles and semen of infected male birds [68]. Bande et al. [3] who reviewed and discussed aspects on pathogenesis and diagnostic methods for IBV infection reported that pathological changes observed grossly at necropsy include congestion and oedema of tracheal mucosa and extrapulmonary bronchi. Histopathological changes include loss of cilia, oedema, rounding and sloughing of epithelial cells, and infiltration by lymphocytes [3]. Some strains of IBV also infect non-respiratory tissues including reproductive tissues [69], kidneys [70][71], and the alimentary tract [9]. IBV was detected in the kidneys in various studies [35][72]. On the other hand, it was reported that renal lesions due to infection with a 793/B-like isolate of virus consisted of tubular degeneration, desquamation and necrosis of the epithelium and an inflammatory cell reaction in the interstitium [62] and it was suggested that the virus has a tropism for the epithelium, especially that of the collecting tubules of the kidney [63]. The IBV was detected in the caecal tonsils and cloaca of chicks (12 days age inoculated intra-ocularly with IBV, 103 EID50 of the 793/B isolate) from the 2nd to the 12th day post-inoculation (PI). The virus was also detected in the kidneys from days 4-10 PI and in the bursa of Fabricius from days 4-12 PI. The virus was detected in the trachea, lungs and thymus. Histopathological changes developed from 2 days PI and were seen in all parts of the kidney and the lesions were constant during this experiment. These changes included congestion, haemorrhage, necrosis of urinary ducts and lymphocytic infiltration [25]. Besides respiratory lesions, early exposure to IBV causes extensive damage to a wide range of epithelial surfaces in various parts of the body [2]. Infectious bronchitis virus has a great economic effect on the layer industry, because it affects egg production. Besides respiratory lesions, early exposure to IBV causes extensive damage to a wide range of epithelial surfaces in various parts of the body [2]. In layers, viral infection at an early age causes permanent damage to the oviduct [73], along with some respiratory signs [13] [14]. The initial and principal site of viral replication is the epithelium of the trachea, where IBV can be detected at day one after infection. From this site, the virus spreads to other internal organs, such as the lung, spleen, liver, kidney, oviduct, ovaries, testes, digestive and intestinal tracts [74]. It is well known that spleen is the biggest peripheral immune organ and plays an important role in immune reaction. Splenic nodules are where B lymphocytes gather and mature, and that the periarterial lymphatic sheath consists of matured T lymphocytes [75]. The distinction between red and white pulp is less sharp than mammals [76]. In the present study, moderate depletion of cellularity in red and white pulp beside reduce splenic corpuscles in the spleen of non vaccinated naturally IBV infected chicken may be indicated suppression of B cell and T cell proliferation that would impaired the deficiency and function of spleen

DOI: 10.9790/2380-1203024857 www.iosrjournals.org 54 | Page
V. Conclusion

The results of this study indicated that infectious bronchitis virus (IBV) induced respiratory and neurological signs, and although IBV primarily affecting the respiratory tract some IBV strains infected many organs such as the lung, trachea, spleen, oviduct, kidney, liver, cerebellum and spinal cord and lead to decrease quality and quantity chicken productions which causes significant effect to the poultry industry in Libya. The necropsy and histopathological examination indicated the involvement of nephropathogenic avian IBV. Avian infectious bronchitis still poses a great challenge for the chicken industry worldwide. As a virus that goes through continuous changes, a large number of regional and global variants have been identified. Therefore, further study is needed to controlled and try to lessened the deleterious effects of infectious bronchitis virus.

Acknowledgements

The authors thank the following individuals for their assistance: Dr. Khaled M. A. Hussin for his contribution in review and format the manuscript.

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DOI: 10.9790/2380-1203024857 www.iosrjournals.org
Serological and Histopathological Studies on Infectious Bronchitis in Non Vaccinated Layers a....


