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Relation between Ascites Syndrome Incidence and Infectious Bronchitis in Broiler Chickens by ELISA Method

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Abstract: Infectious bronchitis is an acute viral disease with high contagious and mortality among chicks. The aim of this study was to survey of relation between ascites syndrome incidence and infectious bronchitis in broiler chickens by ELISA method in Iran. Eight Ross strain broiler farm affected by infectious bronchitis were selected in this study. Blood samples were gathered early stages of disease and blood sampling was repeated two times with seven days interval. ELISA serologic test was used for approving the determination of infectious bronchitis. In addition, in order to differential diagnosis of Newcastle and influenza (H_9N_2) some relevant experiments were conducted. The rate of mortality in any farm during rearing, autopsy and the cause of mortality were recorded. Ascites cases were calculated in terms of prevalence. The growth parameters, FCR, final weight, total consumption of grain at each farm were calculated and mentioned. Based on obtained results in this study, the mean rate of mortality caused by ascites syndrome has been increased meaningfully in herds affected by infectious bronchitis compared with control group. In eight understudied farms affected by infectious bronchitis, the mean rate of Ascites mortality was 3% such that the mean rate of Ascites mortality was 0.5% at previous periods. Based on relevant results also final weight mean in affected herds with infectious bronchitis was lower compared with previous periods. Meanwhile, FCR in affected herds with infectious bronchitis was high compared with healthy herds. In this research demonstrated that there is positive correlation between infectious bronchitis and Ascites syndrome and the correlation is significant ($p<0.05$).

Key words: Ascites syndrome, broiler chickens, ELISA, infectious bronchitis

INTRODUCTION

Infectious bronchitis in poultry is an acute viral disease with high contagion in chicks, which reveal with tracheal rales, coughing and sneezing. The disease causes many economical losses, weight, and feeding rate decrease. Several serotypes that have bronchitis virus cause the cost increase in order to preventing it (Saif *et al*., 2008).

The mortality arising from the disease in broiler poultry is very important considering economical factor. The rate of mortality in broiler poultry typically reaches to its maximum rate at 5 to 6 weeks and its rate increase because of secondary infection. Some viral strains are nephropathologic and cause mortality to 30% in young poultries (Saif *et al*., 2008).

Ascites syndrome means abnormal increasing of endemic transotic fluid in one or more different spaces of abdominal area. The great accumulation of this fluid is seen in liver area especially in hepatoperitoneal (Fig. 1) and in pericardial area. Now, ascites syndrome considered as one of the serious problems in broiler poultry rearing. The syndrome reveals with accumulation of serous in abdominal area. Typically, affects the young and fest growing poultries (Hassanzadeh, 2009). Ascites syndrome occurs all over the world in growing broiler chicks and considered as one of the important mortality causes in broiler herds (Nakamura *et al*., 1999; Riddell, 1985). How ever, there are some reports about suffering guinea fowl, duck, and turkey from the syndrome (Cowen *et al*., 1988; Julian *et al*., 1986; Julian and Wilson, 1986). The syndrome was reported in broiler herds of Bolivia altitudes (altitude more than 1800 m from sea level) (Hall and Machicao, 1968).

To days, occurrence of the syndrome is reported both in high and how altitudes' herds (Buys and Barnes, 1981; Cueva *et al*., 1974; Witzel *et al*., 1990). Based on researchers' findings the most important factor in ascites syndrome occurrence is hypoxia (Maxwell *et al*., 1987; Owen *et al*., 1995; Owen *et al*., 1993). All of infectious diseases of broiler chicks' respiratory system, which cause destruction of pulmonary tissue, will lead the reduction of respiratory capacity and hypoxia so with increasing pulmonary blood pressure produce ascites syndrome (Hassanzadeh, 2009). In some diseases like CRD, infectious bronchitis, coli bacillus and aspergillus that

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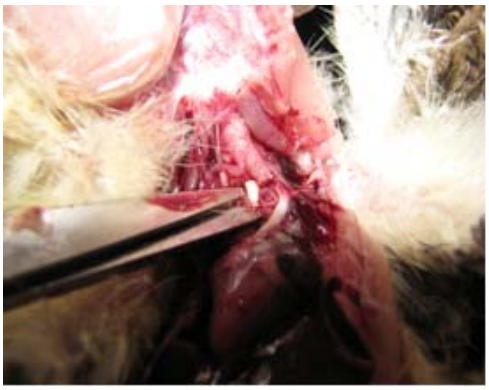

Fig. 1: Accumulation of Ascites fluid contained fibrin in abdominal area and edematous liver in Ascites syndrome

the pulmonary tissue is destroyed and its capacity reduces, the occurrence of ascites syndrome is decisive (Cook *et al*., 1986; Darbyshire, 1985; Ganapathy and Bradbury, 1999; Hofstad and Yoder, 1996; Julian and Goryo, 1990; Lucio and Fabricant, 1990). Marius *et al*. (2009) showed respiratory system damages after the coincident infectious with *E. coli* and bronchitis infectious virus in broiler chicks. In affected birds by these two microbes also air sacs' damages was seen (Marius *et al*., 2009).

Zafra *et al*. (2008) demonstrated the occurrence of ascites syndrome following fungal aspergilusis disease, which has led to the destruction of pulmonary tissue (Zafra *et al*., 2008). Enkvetchakul showed the effect of inflammatory reactions on increasing the thickness of gas exchange area in lungs. In relation with this issue, some infectious agents like aspergillus, *E. coli* and bronchitis virus have been mentioned (Enkvetchakul *et al*., 1995). Wideman *et al*. (1997) proved the relationship between the increased tolerance to blood circulation in pulmonary and ascites syndrome (Wideman *et al*., 1995; Wideman and Kirby, 1995; Wideman *et al*., 1997). Regarding that the ascites syndrome considered as one of the major economical problems, controlling actions must be done about the problem. The most important controlling program is identifying the ascites syndrome caused factors. Because infectious bronchitis intensifies ascites syndrome occurrence by reducing pulmonary capacity, the study aimed at determining the rate of this relationship. By this way, we can understand accurately the importance of infectious bronchitis prevention, which assists in preventing the ascites syndrome.

MATERIALS AND METHODS

This study was conducted in important farms of east Azerbaijan province, Iran during May-August 2011. Eight Ross 308 strain broiler farm affected by infectious bronchitis were selected for the study. Their disease diagnosed by clinical signs, autopsy, and serologic signs. In all affected farms, clinical signs like respiration with open mouth (gasping), focal accumulation, and lack of movement were observed. Trachea hyperemia, caseous exotic accumulation in trachea branching and pulmonary hyperemia was observed in autopsy.

Table 1: Feeding diet in understudying farms

	Age			
Foodstuff	0 - 20th day (Kg)	21-35 (Kg) (Kg)	36-42 (Kg)	43th day to selling time (Kg)
Corn	555	590	630	670
Soybean	370	330	290	250
Meat concentration	50	50	50	50
Oyster	15	15	15	15
Oil	10	16	16	16
Cleanacooks	200 g	200 g	200 g	200 g

*: 0.5 concentrations from Goldenbro co. were used

At the begging of affecting (usually, days of 18 to 28) blood samples were gathered and then two times resampling by 7 days intervals was done. Fifteen serum samples of each farm were selected. In order to determine the rate of serum antibody against infectious bronchitis and to differential diagnosis of influenza and New castle disease, ELISA serologic test (using IDEXX kit) and HI serologic test were used, respectively and their results are given in Results section.

The rate and cause of mortality at each farm during rearing period was recorded until 50th day and autopsy was conducted. The ascites syndrome cases were autopsied carefully and its prevalence was calculated. Regarding ascites mortality, the following were considered: heart dilatation especially calculation of the ratio of right ventricle weight to whole heart, hydropericardiac, transotic fluid accumulation in abdominal area.

It must be mentioned that comparative analysis of ascites syndrome in any affected infectious bronchitis farm was conducted compared with pervious periods that don't affected by the disease and were observed in the regard of rearing, feeding and vaccination condition.

Radiation of light: Radiation of light was identical in all affected farms and consisted of 23 h light and one hour without light.

Density: The rate of density in all farms was 10 birds per 1 square meter.

Feeding program: All farms have identical feeding program that is showed in Table 1.

Vaccination program: Vaccination program at all understudied farms was identical as following:

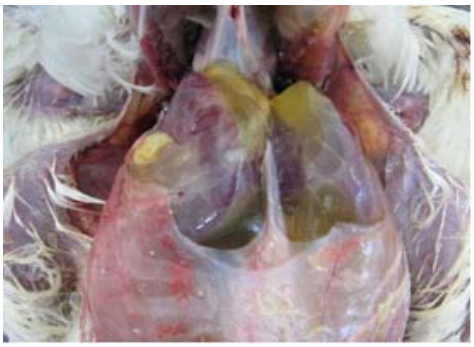

Fig. 2: Caseous suppuration of tracheal branching in infectious bronchitis disease

Fig. 3: Pulmonary sever hyperemia in Ascites syndrome

Fig. 4: Right ventricle hypertrophy in Ascites syndrome

- **First day:** Bronchitis vaccine (H_{120}), spray form
- **Tenth day:** Injecting vaccine of Newcastle + influenza along with B_1 vaccine, eye drops
- **Fourteenth day:** Gambro vaccine (Bursine-2), oral
- **Eighteenth day:** Newcastle vaccine (Clone), oral Twenty-first day: Gambro vaccine (Bursine-2), oral
- **Thirteen day:** Newcastle vaccine (Clone), oral

Evaluating the growth parameters: FCR, final weight and total consumption of grain at each farm was calculated and recorded in order to evaluation of growth parameters. It is noteworthy that FCR is obtained by calculating total consumption of grains and its division to alive weight of total herd in each of understudied farms.

Right Ventricular / Total Ventricular Ratio (RV/TV): After the autopsy, the heart of individual chicks separated from the corpse and large blood vessels, sinuses, atriums and the fat around heart was removed. Then right ventricle was separated from its connection to septum between two ventricles. The blood of ventricles was removed and ventricles were rinsed. The ratio of right ventricle to total ventricular was identified by calculating right ventricle weight and two ventricular using sensitive scales. If the ratio was more than 29%, it would be considered as right ventricle hypertrophy and ascites and if the ratio was less than 25% it would be considered as normal and unascites heart.

Statistical calculations: In this study, all of statistical calculations were conducted using SPSS software (version 17), T-test, and correlation.

RESULTS

Relevant findings of eight broiler farms affected by infectious bronchitis which diagnosed by clinical signs (Fig. 2-4), autopsy and serologic data (HI for New castle and Influenza) are mentioned as follows:

The results of ELISA test for infectious bronchitis and HI test for new castle and influenza were used for statistical calculations:

Total mortality: In affected herds by infectious bronchitis that have been infected under sixth week, clinical signs and autopsy results were recorded. Respiration with open mouth, swelling of eyes, darkness of comb and whiskers, crowding, respiratory reactions, reduction of grain consumption, and in some extent decrease in water consumption were observed as clinical signs. Tracheal hyperemia, caseous suppuration in tracheal branching, and pulmonary hyperemia are the most important signs of autopsy. Also other mortality factors were assessed in understudied herds and total mortality was calculated that is shown in Table 3.

As the results showed in Table 2, broiler farms affected by infectious bronchitis have increased antibody titers in three-times of blood sampling for differential diagnosis of New castle and Influenza and this increased rate associated with vaccination program whereas increased rate of antibody titers of infectious bronchitis in three-times of blood sampling associated with infectious bronchitis. Autopsy results, of course, conform to increased antibody titer of infectious bronchitis.

Based on results obtained from t-test the mean percentage of mortality caused by Ascites syndrome in

Table 2: Comparative analysis of antibody titers of infectious bronchitis, influenza, and new castle in affected herds by infectious bronchitis and control groups

	Antibody								
Titers farm	IB First time	IB Second time	IB Third time	Al First time	Al Second time	Al Third time	ND First time	ND Second time	ND Third time
Affected group by bronchitis (1)	892	1786	3689[a]	1.8	2.8	3.6	2.5	3.1	4.2
Control group (1)	851	1210	1350[b]	1.6	2.6	3.2	2.4	3.3	4.7
Affected group by bronchitis (2)	924	1524	4002[a]	2.1	3	3.5	1.9	2.7	4.6
Control group (2)	937	1120	1180[a]	2.3	2.5	3.9	2.2	2.8	5.2
Affected group by bronchitis (3)	900	1358	3 877	1.5	2.6	4	2.1	3.3	5
Control group (3)	892	1050	1105[b]	1.8	2.4	3.3	2.5	3	5.5
Affected group by bronchitis (4)	950	1315	3508[a]	2.1	2.7	4.1	2	2.9	4.3
Control group (4)	920	1125	1210[b]	2.4	2.6	4.1	1.9	2.5	5.1
Affected group by bronchitis (5)	1099	1409	3683[a]	1.6	2.4	3.9	1.7	2.5	4.3
Control group (5)	1001	1210	1510[b]	1.9	2.9	4.5	1.8	2.4	4.9
Affected group by bronchitis (6)	819	2114	4568[a]	2.8	3.6	4.1	2.8	3.1	4.6
Control group (6)	1010	1310	1620[b]	1.9	2.8	3.9	2.3	2.7	5.5
Affected group by bronchitis (7)	800	1480	4640[a]	2	2.6	3.5	2.1	2.7	4.4
Control group (7)	970	1180	1300[b]	1.5	2.3	4.2	1.8	2.5	5.1
Affected group by bronchitis (8)	844	1588	4660[a]	1.7	2.6	3.4	1.8	2.7	4.7
Control group (8)	817	1020	1070[b]	2.1	3	3.5	1.5	2.8	5.2

*: Determining antibody titer of bronchitis with ELISA and Newcastle and influenza with HI test; Means within a column with different superscript letters (a, b) denote significant differences ($p<0.05$)

Table 3: Comparative examination of mortality factors in groups affected by infectious bronchitis and control groups

Total mortality (%)	Ascites syndrome (%)	Coli bacillosis (%)	Infectious bronchitis (%)	yolk sac (%)	Trial group	The age of infection in affected groups	Number	Farm
1.8[a]	3[a]	A 5.5	8	1.5	Affected group by infectious bronchitis	18 days	10000	1
6_b	0.5[b]	4.5[b]	0	1	control group			
14.8[a]	3.3[a]	4.5[a]	6	1	Affected group by infectious bronchitis	21 days	5000	2
5[b]	0.6[b]	3.2[b]	0	1.2	control group			
17[a]	3.1[a]	5.4[a]	7	1.5	Affected group by infectious bronchitis	23 days	30000	3
7[b]	0.8[b]	4.1[b]	0	1.4	control group			
19	2.6[a]	5.4[a]	9	2	Affected group by infectious bronchitis	26 days	10000	4
6.5[b]	0.7[b]	4.1[b]	0	1.7	control group			
14.7[a]	3a	5.2[a]	5	1.5	Affected group by infectious bronchitis	24 days	14000	5
5.5[b]	0.4[b]	3.6[b]	0	1.5	control group			
19. 5[a]	2.8[a]	5.5[a]	10	1.2	Affected group by infectious bronchitis	28 days	7000	6
6[b]	0.2[b]	4.4[b]	1	1.4	control group			
18.8[a]	3.2[a]	5.5[a]	8	1.6	Affected group infectious bronchitis	26 days	9000	7
7[b]	0.3[b]	4.2[b]	0	1.2	control group			
17.1[a]	3a	4.5[a]	8.1	1.5	Affected group by infectious bronchitis	23 days	11000	8
6[b]	0.5[b]	3.1[b]	0	1.4	control group			

Means within a column with different superscript letters (a, b) denote significant differences ($p<0.05$)

control group was less than the group affected by infectious bronchitis that, were 0.54±0.11 and 3.05±0.44 respectively (Mean±SE).

There is meaningful difference between control group and affected group in mortality percentage caused by ascites syndrome using t-test ($p<0.05$) ($p = 0.001$).

Table 4: Comparison of percentage mean of mortality caused by ascites syndrome in two trial groups

Mortality percentage caused by ascites syndrome	Group	Farm number	Mean	S.D	MSE
	Control group	8	0/5475[a]	0/32376	0/11447
	Affected group by bronchitis	8	3/0575[b]	½5619	0/44413

Means within a column with different superscript letters (a, b) denote significant differences ($p<0.05$)

Table 5: Comparison of final weight between two trial groups

Body final weight	Group	Farm number	Mean	S.D	MSE
	Control group	8	2550/1250[a]	100/68542	35/59767
	Affected group by bronchitis	8	2347/1250[b]	46/56926	16/46472

Means within a column with different superscript letters (a, b) denote significant differences ($p<0.05$)

Comparing the mean of final weight between two trial groups: Based on results obtained from t-test the mean of body final weight in control group was more than the group affected by infectious bronchitis, that were 2550.12±35.59 and 2347.12±16.46, respectively (Mean± SE).

There is meaningful difference between control group and affected group in body final weight using T-test ($p<0.05$) ($p = 0.001$).

Comparing the mean FCR between two trial groups: Based on results obtained from T-test the mean of FCR in control group was less than the group affected by infectious bronchitis, that were 2.000±0.01 and 2.25±0.02 respectively (Mean ± SE).

There is meaningful difference between control group and affected group in FCR using t-test ($p<0.05$) ($p = 0.001$).

Results Obtained from Comparing Ventricle Weights Ratio (RV/TV): After the autopsy, the heart of individual chicks separated from the corpse and large blood vessels, sinuses, atriums and the fat around heart was removed. Then right ventricle was separated from its connection to septum between two ventricles. The blood of ventricles was removed and ventricles were rinsed. The ratio of right ventricle to total ventricular was identified by calculating right ventricle weight and two ventricular using sensitive scales and results are as follows:

Based on results obtained from t-test the mean of (RV/TV) in control group was 24.00±0.5 and in the affected group by infectious bronchitis was 29.25±0.21 (Mean ± SE) and the difference is so meaningful statistically ($p<0.05$) ($p = 0.001$).

Determining the relationship between infectious bronchitis and the rate of mortality caused by ascites syndrome: There is positive correlation between infectious bronchitis and mortality rate caused by ascites syndrome (Pearson Correlation = 0.826) and this correlation is very meaningful. It must be note that the rate of mortality caused by ascites syndrome increases with increasing infectious bronchitis disease, the rate of mortality caused by ascites syndrome increases, too.

DISCUSSION AND CONCLUSION

Nowadays, ascites syndrome is one the important factors of broiler poultries' mortality (Nakamura *et al*., 1999). The syndrome was reported for the first time in reared herds in Bolivia high altitudes (Hall and Machicao, 1968). In any case, ascites syndrome, also, considered as one of the important problems in low altitudes because of fast growth of broiler chicks (Buys and Barnes, 1981; Cueva *et al*., 1974; Witzel *et al*., 1990). Regarding to high economical importance of the syndrome; in order to control causing effects, it is necessary to investigate and prevent all of affecting factors.

One of the factors causing ascites syndrome is hypoxia condition (Maxwell *et al*., 1987; Owen *et al*., 1995; Owen *et al*., 1993; Shlosberg *et al*., 1992).

Generally, any factor that intensifies hypoxia will increase the mortality caused by ascites syndrome (Mirsalimi and Julian, 1991).

Inflammatory reactions lead to increase thickness of gas exchange area that may even remain after the removal of causal factor (Enkvetchakul *et al*., 1995). With regard to the issue, infectious factors like aspergillus, Escherichia coli, and infectious bronchitis virus cause to pulmonary damage, right ventricle hypertrophy followed by ascites syndrome (Tottori *et al*., 1997). In fact, the etiology associated with this issue relates to increased tolerance to pulmonary blood circulation, which in turn causes pulmonary high blood pressure followed by right ventricle insufficiency and ascites syndrome (Wideman *et al*., 1995; Wideman and Kirby, 1995). All of infectious diseases of broiler chicks' respiratory system that cause pulmonary tissue destruction and reduction of respiratory capacity, can lead to oxygen deficiency and increased level of pulmonary blood pressure and ascites by reducing the volume of gas exchanges (Hassanzadeh, 2009). In present study, infectious bronchitis, as a destroying factor of pulmonary tissue, has caused to reduce the pulmonary respiratory capacity and hypoxia. The birds' lung has less

Table 8: Correlation between the rate of affecting by infectious bronchitis and the rate of mortality caused by ascites syndrome

		The rate of bronchitis	Mortality rate caused by ascites
The rate of bronchitis	Pearson correlation	1	0/826**
	Sig.(1-tailed)		0/000
	N	16	16
Mortality rate caused by ascites	Pearson correlation	0/826**	1
	Sig.(1-tailed)	0/000	
	N	16	16

**: Correlation is significant at the 0.01 level (1-tailed)

Table 6: Comparing the mean FCR between two trial groups

FCR	Group	Farm number	M.D	S.S.D	Mean
	Control group	8	2/0088[a]	0/04824	0/01705
	Affected group by bronchitis	8	2/2588[b]	0/06105	0/02158

Means within a column with different superscript letters (a, b) denote significant differences (p<0.05)

Table 7: Comparison the mean ratio of right ventricle weight to total ventricle (RV/TV) between two trial groups

(RV/TV)	Group	Farm number	M.D	S.D	MSE
	Control group	8	24/0000[a]	1/41421	0/50000
	Affected group by bronchitis	8	29/250[b]	00/59761	0/21129

Means within a column with different superscript letters (a, b) denote significant differences (p<0.05)

flexibility compared with mammalians' one and their capillaries also have weaker dilation (Hassanzadeh, 2009); therefore, respiratory infections of broiler poultry can weaken respiratory system's efficiency followed by hypoxia.

Based on results obtained from Table 4, in this study the mean percentage of mortality caused by ascites syndrome in affected herds by infectious bronchitis has increased meaningfully compared with control herds (p<0.05) and the results have confirmed by comparison with other studies. (Hassanzadeh, 2009; Enkvetchakul *et al*., 1995; Tottori *et al*., 1997). In eight-understudied farm that affected by infectious bronchitis and in healthy herds the mean ascites mortality was 0.3% and 0.5%, respectively; that the increased rate of ascites mortality in affected by infectious bronchitis was six times more than control group. Then we find that infectious bronchitis is considered as an important factor in ascites syndrome occurrence. Further, the results obtained from Table 5 shows that final weight in affected herds by infectious bronchitis was less than healthy herds, meaningfully (p<0.05). These results demonstrate the effect of respiratory diseases on growth. Affected herds by infectious bronchitis have decreased pulmonary capacity, so aerobic oxidation is disordered and the chicks become sensitive to secondary infections; therefore, this factor has direct influence on final weight. Based on results obtained from Table 6, FCR in affected herds by infectious bronchitis was more compared with healthy herds (p<0.05). Regarding that, one of the important factors in growth is supplying oxygen and aerobic oxidation, metabolism process is disordered in affected herds by infectious bronchitis because of pulmonary deficiency and lack of oxygen supplying; so have negative effect on metabolism. Based on results obtained from Table 8, there is positive correlation between infectious bronchitis and ascites syndrome occurrence and the correlation is very meaningful (p<0.05). based on results obtained from Table 7, the mean ratio of right ventricle to total ventricle (RV/TV) in affected herds by infectious bronchitis and ascites syndrome has increased meaningfully compared with healthy herds (p<0.05). The mean in affected herds by ascites syndrome was more than 29% and in healthy herds was about 24%; that is meaningful difference in statistical analysis (p<0.05). Our findings conform to findings of other researches. (Julian, 1989; Julian *et al*., 1987; Julian *et al*., 1993; Van vleet, 1986).

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