DETECTION OF CILIARY ACTIVITY FOLLOWING VACCINATION WITH 3 COMMERITIAL INFECTIOUS BRONCHITIS VACCINES IN BROILER BIRDS

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ABSTRACT

This study was conducted to investigate whether different types of Infectious Bronchitis Virus vaccines can affect the cilia of tracheal epithelium of broiler birds, in comparison with Newcastle Disease and Avian Influenza Vaccines. as positive control. Sixty one-day-old broiler birds of both sexes were used in this experiment. The bird were randomly divided in to six groups of ten birds each. Each group was placed in a separated pin.One of these groups,Group 6 was acted as negative control,whereas the other 5groups were vaccinated with ND, AI and 3 type of IB vaccines. Age, dose and route of administration of different type of vaccines were conducted according to the manufactures directions. Group 1 was vaccinated with ND vaccine at seventh & seventeenth day of age , group 2 was vaccinated with AIV vaccine at first & twenty one day of age , these 2 groups were acted as positive control .Group 3,4,5, were vaccinated at first&tenth day of age with different types of IB vaccines . BIVAC1 was used for group 3, IBMA5 was used for vaccination of group 4, whereas IB-H120 vaccine was administrated to group 5 . Four days post vaccination; all groups were observed for clinical signs. All experimental birds were killed and tracheal rings were examined for ciliostasis and Carboniferous Pigment Granules test. The result indicated that group ND vaccineswas 95%, while that of group AI vaccines was 93% whereas the results of these 3 types of vaccines were 21%, 33%, 20% for BIVAC1, IBMA5 &IBH120 respectively, while the control was 100% .

INTRODUCTION

The upper respiratory system in the nasal cavity is well designed to heat, humidify and filter the inspired air. The expanded, mucous-covered epithelial surfaces possess cilia, where inspired particulate material may impact, and eliminated outside (1),(2).

The trapped particles can be rapidly removed from the nasal cavity Also the effectiveness of this site in the respiratory system in preventing microorganisms in the air stream from entering the trachea and lower parts of the respiratory system. (3).

Mucociliary clearance plays a pivotal role in defending the respiratory system, from the nose and upper airways to the lower tract .The efficiency of mucociliary clearance depends on the balance and integrated coordination of three components, including the volume and composition of the airway surface liquid (mucus and periciliary fluid), ciliary structure and ciliary beating frequency, and mucus–cilia interaction (4),(5) .

The airway surface liquid traps inhaled particulates and microorganisms, which are continuously transported by the ciliary activity towards the oropharynx, where they are swallowed or expectorated. An imbalance in one or more components leads to impaired mucociliary clearance, which is associated with increased susceptibility to respiratory infection (6),(7).

The ciliated epithelial lining of the pulmonary tract plays a vital role in the body's defines against disease by removing inspired foreign particles from the respiratory tract, Effective ciliary activity is dependent upon proper mucus viscosity, mucus pH, ciliary beat frequency and ciliary beat synchrony (8).

Cilia on respiratory epithelial cells are responsible for movement of fluid and particles over the cell surface (9).

Ciliary dysfunction can lead to an increased susceptibility to chronic respiratory infection and distress (10).

Several methods have been described to assess mucociliaryfunction (11).

This experiment was conducted to investigate the effect of IB virusvaccines on tracheal cilia broiler birds through examination of ciliary activity by direct light microscope and Carboniferous Pigment Granules movement.

MATERIALS AND METHODS

This experiment was conductedto investigate whether different types of Infectious Bronchitis Virus vaccines (IBV) can affect the cilia of tracheal epithelium of broiler birds, in comparison with ND and AIV Vaccines which has been known that have no adverse effect on the cilia (positive control), as well as unvaccinated negative control group .

Atotal of sixty one-day old Ross broiler chicks were used in this study. The birds were raised in an isolated cage in the Experimental House of the Department of Pathology and Poultry Diseases, College of Veterinary Medicine, Basra University under strict hygienic and standard management conditions. Pellet feed and water were supplied ad-libitum during the interval of the experiment.

The chicks were delivered from Fadac Hatchery at Basrha Province. They were reared until the end of the experiments. Trachea of each bird was examined for ciliary activity .Each trachea was removed with minimum trauma, washed by shaking in normal saline for removal mucous secretion and then transverse sections very thinly (approximately 1.5 mm thick) using a surgical blade to prepare tracheal rings. Two rings were prepared from the upper and lower parts of the trachea and one from the middle portion, making a total of 5 sections from each bird. Each ring was placed individually in tissue culture tubes containing Dulbecco s modified eagle medium (DMEM) and stored in incubator at 37 C. (12) .

Ciliary activity in the excised chicken trachea was maintained for several hours when the tissue was provided with an atmosphere of high humidity, a constant temperature of 38°C , and a continuous source of fluid suitable to Simulate the natural mucus blanket in which the cilia function in the intact animal (13) .

Their ciliary movement was then observed microscopically and the rings were within DMEM by putting Petri dishes under light microscope and scored on a scale from zero (0% ciliary activity) to four (100% ciliary activity) , Percentage of the ciliostasis score was calculated using the following formula where: CS1 – mean ciliostasis score for vaccinated group; CS2 – mean ciliostasis score for corresponding no vaccinated group. (14).

$$\frac{cs1}{cs2} \times 100 \quad [\qquad\qquad]$$

Ciliostasis test was carried out as described by (15) briefly as follows:

Five birds from each group were used for this purpose, 4-7 days post vaccination five thin tracheal rings (2 from the top and bottom and 1 from the middle of the trachea) were selected from each bird , the rings were observed for ciliary activity using a light microscope .The level of beating of the cilia for each ring was expressed as 0 (0% of cilia beating , total lack of protection),1(0-25% beating), 2 (25-50% still beating) , 3 (50 – 75% beating) or 4 (75 – 100% beating) . An individual chick and groups were recorded as protected against challenge if ciliostasis score 50% or more ciliary activity (14).

Scoring the activity of tracheal cilia as a main index of vaccine protectionTable (1) (12).

Table (1): Ciliostasis test:

score	Ciliary Activity
0	**0% Cilia activity , non-beating cilia , lack of protection**
1	**25 % Cilia beating**
2	**50% Cilia beating**
3	**75% Cilia beating**
4	**100% Ciliary activity , all beating , full protection**

Carboniferous Granules Pigment test was also used for detection of ciliary activity .Three milligrams per bird of Carboniferous Pigment Granules were inserted into thoracic inlet of trachea of birds and whole body of them was placed into wet chamber for 45 minutes at 37°C.

To evaluate ciliary activity, the pigments movement in trachea was observed visually (16).

Vaccines and vaccination:

Vaccines:

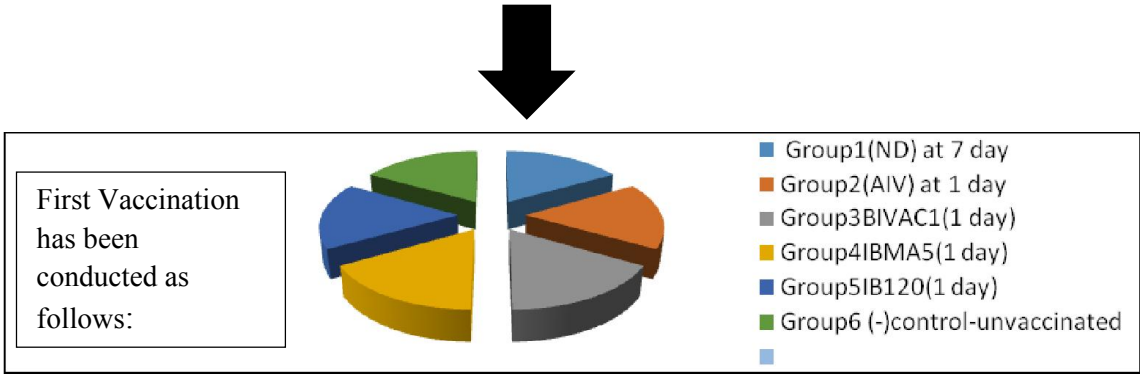
Three different commercially available vaccines against IB were used, each Produced by Internet, International .These were H-120 Vaccine: (1000 doses) per vial, Ma5 Vaccine: (1000 doses) per vial, of the Massachusetts serotype and BI-VAC1˚:(1000 doses) pervial. They were administered at the Manufacturer's recommended dose by the oculonasal (o.n.) route or spray or drinking water according to experimental design .

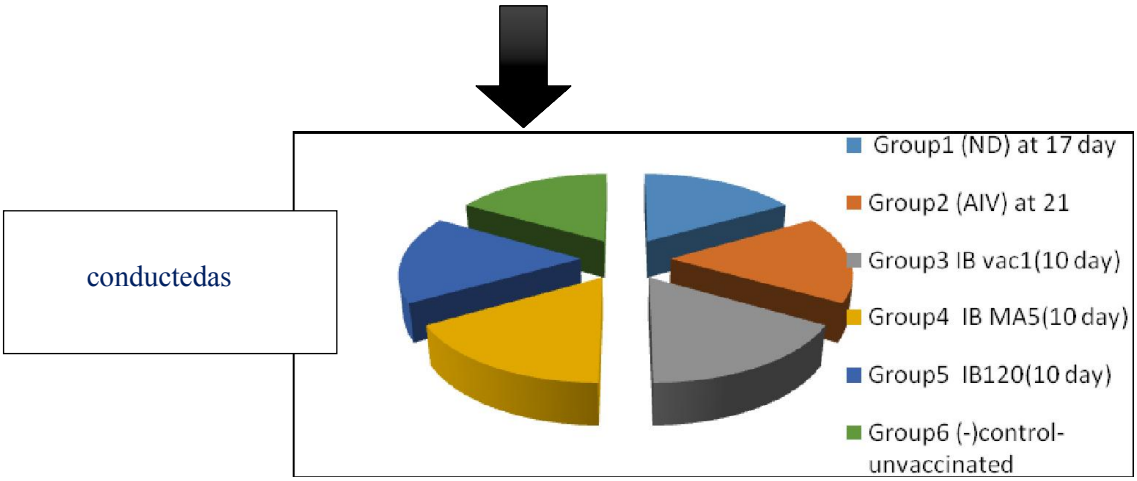
Table (2) : Protection Vaccination Schedule during the Experiments .

Type of Vaccine	First Vaccination	Second Vaccination	Route of administration
Infectious Bronchitis	1 – day	21 – day	Eye drop
Infectious Bursal Disease (Volvac)	14 – day	Non	Drinking water
Newcastle Disease (Izovac)	7 – day	17 – day	Eye drop
Avian Influenza (H9N2)	1 – day	21 – day	Subcutaneous

Experimental Design :

Six groups of one – day old, broiler birds each consist of 10 birds were used in this study





Group 1&2 were acted as positive control.

Group 6 was negative unvaccinated group.

Detection of clinical signs & ciliary activity 4 days after second vaccination

Via

Carboniferous Pigment Granules Microscopically

Sixty one-day-old broiler birds of both sex were used in this experiment. The bird were randomly divided into six groups of ten birds each. Each group was placed in a separated pin.

One of thses groups (G6) was acted as negative control , whereas the other 5groups were vaccinated as indicated by Table 3 below . Age, dose and route of administration of different type of vaccines were conducted according to the manufactures direction .

Group 1 was vaccinated with ND vaccine at seventh& seventeenth day of age , group 2 was vaccinated with AIV vaccine at first&twenty one day of age , these 2 groups were acted as positive control .Group 3,4,5, were vaccinated at first&tenth day of age with different type of IB vaccines . BIVAC1 was used for group 3 , IBMA5 was used for vaccination of group 4 , whereas IB-H120 vaccines was administrated to group 5 . Four days post vaccination , all groups were observed for clinical signs .All experimental birds were killed by cervical dislocation .And examined for ciliostasis and Carboniferous Pigment Granules test.

Table (3): Showed the design of the Experiment.

Group	Type of vaccine	First vaccine	Second vaccin	Route of administration
1	ND	7-Day	17-day	Eye drop
2	AIV (H9N2)	1-day	21-day	Subcutaneous
3	BI-VAC1	1-day	10-day	Eye drop
4	IBMA5	1-day	10- day	Eye drop
5	IB-H120	1-day	10-day	Eye drop
6	CONTROL	Unvaccinated	------	

- Number of birds in each groups was 10 birds .

RESULTS

A total of sixty one-day old broiler birds have been used in this study , and 150 tracheal rings were examined to detected ciliary movement (5 rings per birds per slide)by light microscope whereas 30 birds were examined by Carboniferous Pigment Granuolus .

The aim of present study was to assess ciliary activity following vaccination with infectious bronchitis virus vaccines, influenza virus vaccine and Newcastle virus vaccine by using Carbon Pigment Granules as rapid methods of test. Prior to vaccination, all chicks were apparently normal .

The result of group four, which was actedas negativecontrol, no clinical signs were observed during their entire trial period. After inserting pigment granules in the lower part of trachea , the birds in this group showed normal ciliary activity .

The pigment granules moved up and reached larynx area during 45 minutes post insertion. Post-vaccination, the birds which have been vaccinated with the three IB vaccine (IBH120, IBMA5&BIVAC1) showed moderate clinical signs such as tracheal rales and nasal discharge .After pigment insertion, in trachea of birds in IB vaccinated groups,pigment granules did not move up normally. Some cases in these groups revealed that movement of pigments was apparent for small distance.

Pigments granules did not appear to be transported along trachea to the larynx, whereas AI and ND vaccinated birds , pigment granules were appeared to transport along trachea the larynx through 45 minutes after insertion of pigments granules,as indicated in Table 4.

Table (4): Clinical signs and pigment test results:

Group	Type of vaccine	Clinical signs	Pigments	%
1	ND	0	5	0/100
2	AI	0	5	0/100
3	BIVAC1	6	0	60/0
4	IBMA5	6	0	60/0
5	IB-H120	5	0	50/0
6	Control	0	5	0/100

- 10 birds were used in each group . they were observed for evidence of clinical signs , then they were divided into 2 sub-groups , one for pigment test and the other for ciliostasis .

Ciliostasis test:

Table (5) Ciliary activity of group 1 vaccinated by Newcastle Disease virus vaccine at seventh day of age and revaccinated at seventeenth day of age . Score: 0 = no activity; 4 = 100% activity.

Group 1	Bird no	Tracheal rings 1	2	3	4	5	Average Score	% Protection
ND	1	4	4	4	4	4	4	100
	2	3	4	4	4	4	3.8	95
	3	4	4	3	4	4	3.8	95
	4	4	4	4	3	4	3.8	95
	5	4	3	4	4	3	3.6	90
total							**3.8**	**95%**

Table (6) Ciliary activity of group 2 vaccinated by Avian Influenza virus vaccineatfirst day of age and revaccinated at twenty one day of age . Score: 0 = no activity; 4 = 100% activity .

Group 2	Bird no	Tracheal rings 1	2	3	4	5	Average Score	% Protection
AIV	1	4	4	4	3	4	3.8	95
	2	3	4	4	4	4	3.8	95
	3	4	3	4	4	4	3.8	95
	4	3	4	4	3	4	3.6	90
	5	3	4	4	4	3	3.6	90
total							**3.72**	**93%**

Table (7).Ciliary activity of group 3 vaccinated by strain BIVAC1 at one day andrevaccinated at ten day of age. Score: 0 = no activity; 4 = 100% activity.

Group 3	Bird no	Tracheal rings 1	2	3	4	5	Average Score	% Protection
B1VAC1	1	2	1	1	1	2	1.4	35
	2	1	1	1	1	1	1	25
	3	1	1	1	0	1	0.8	20
	4	1	1	1	1	1	1	25
	5	0	0	0	0	0	0	0
total							**0.84**	**21%**

Table (8).Ciliary activity of group 4 vaccinated with strain IBMA5 at one day and revaccinated at ten dayof age . Score: 0 = no activity; 4 = 100% activity.

Group 4	Bird no	Tracheal rings 1	2	3	4	5	Average Score	% Protection
IB- MA5	1	1	2	1	2	2	1.6	40
	2	1	1	1	1	1	1	25
	3	2	0	1	1	1	1	25
	4	2	1	2	2	2	1.8	45
	5	2	1	0	2	1	1.2	30
total							**1.32**	**33%**

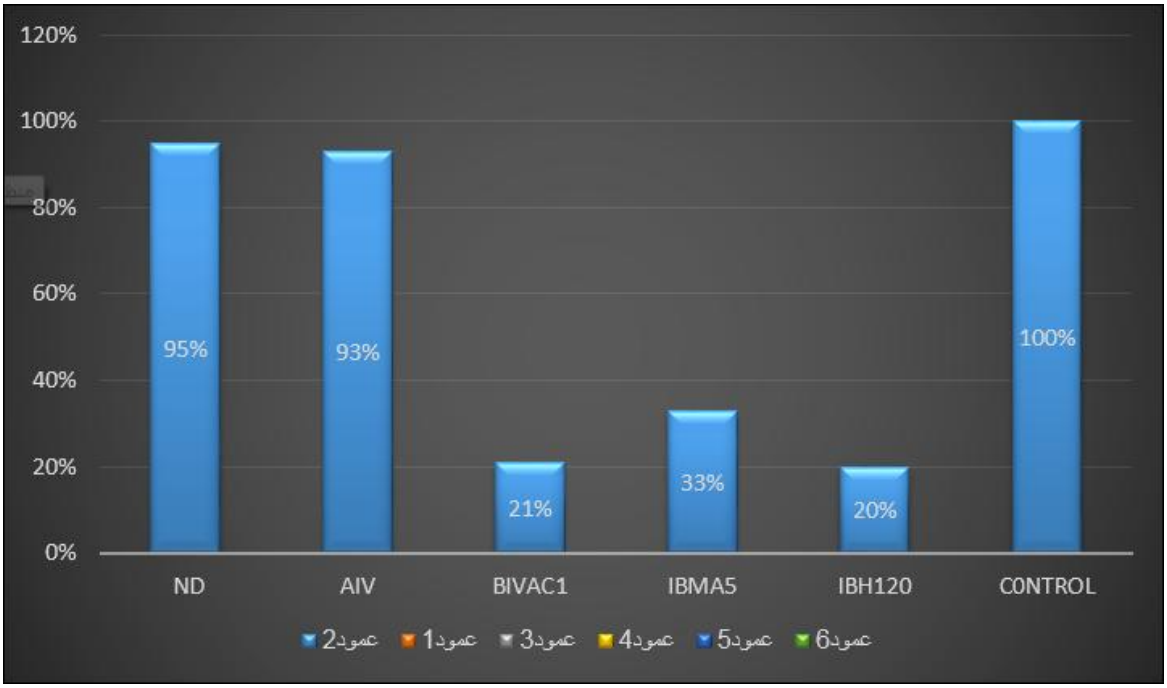
Table (9).Ciliary activity of group 5 vaccinated with strain IB-H120 at one day and revaccinated at ten day of age. Score: 0 = no activity; 4 = 100% activity.

Group 5	Bird no	Tracheal rings 1	2	3	4	5	Average Score	% Protection
IB-H120	1	1	1	1	1	1	1	25
	2	3	2	1	0	3	1.8	45
	3	1	1	2	0	0	0.8	20
	4	0	0	1	0	0	0.2	5
	5	1	0	0	0	0	0.2	5
total							**0.8**	**20%**

Table (10) Ciliary activity of group 6 unvaccinated. Score: 0 = no activity; 4 = 100% activity .

Group 6	Bird no	Tracheal rings 1	2	3	4	5	Average Score	% Protection
Control	1	4	4	4	4	4	4	100
	2	4	4	4	4	4	4	100
	3	4	4	4	4	4	4	100
	4	4	4	4	4	4	4	100
	5	4	4	4	4	4	4	100
Total							4	**100%**

Figure (1): Drowning summarized the results of effect of IB vaccines of this experiment.



DISCUSSION

Abdullah(17). found that attenuated live vaccines may be regain their virulence and the risk is even greater with viruses that have high frequencies of mutations .

The result of the this experiment displayed that the 3 types of IB vaccines strains were exert a clear negative effect on ciliary movement . Microscopically, the results of these 3 types of vaccines were 21% , 33% , 20% for BIVAC1, IBMA5 &IBH120 respectively as showen in table (7 ,8 ,9) and Figure (1) . Whereas Carbon Pigment Granules was not move up from the site of insertion, which indicated that IB vaccines were hinderedthe movement of tracheal cilia.

This result was in agreement with that of(18) who stated that although IB vaccine viruses do not seem to be virulent, they are still able to replicate in respiratory epithelia and induce negative effect on cilia.

Interestingly(19) found that not only the virulent IBV M41 field strain enhanced susceptibility to subsequent *E. coli* infection, but the mild H120 vaccine strain also induces this enhancement for colibacillosis to about the same level.

Decreased ciliary activity and mucociliary clearance caused bacterial superinfection (20). (21) Indicated that ciliary activity is an accurate measure of immunity or cross immunity to avian IBV strains.

IBV could provoke ciliostasis in the host's ciliated airways and may therefore facilitate the opportunity for other related pathogens to induce their pathogenicity (22).

Assessment of ciliary activity has been used to determine safety and efficacy of different strains of infectious bronchitis virus vaccines (23).

These results suggest that Carboniferous Pigment Granule and Ciliostasis test are non-costly, simple and fast approach to observe tracheal ciliary activity in clinical specimens and can be used for rapid differentiation between IBV, ND and AIV infection.

CONCLUSION

In conclusion, this experiment demonstrated that all 3 commercial IB vaccines were exert a clear negative effect on ciliary activity at different degrees of ciliostasis .

الكشف عن النشاط الهدبي بعد التطعيم بثلاث لقاحات تجارية لالتهاب الشعب الهوائية المعدي في افراخ فروج اللحم

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الخلاصه

أجريت هذه الدراسة لمعرفة ما إذا كانت لقاحات فيروس التهاب الشعب الهوائية المعدية تؤثر على أهداب ظهارة القصبة الهوائية في افراخ فروج اللحم ، بالمقارنة مع لقاح النيوكاسل ولقاح انفلونزا الطيورالتي كانت سيطرة ايجابية بالاضافه الى سيطره سلبيه (غير ملقحه). وقد استخدمت في هذه التجربة ستين من الطيور بعمريوم واحد من كلا الجنسين. تم تقسيم الطيور عشوائيا إلى ست مجموعات كل مجموعه عشرة طيور لكل منها. وضعت كل مجموعة في حاجز منفصل. استخدمت واحدة من هذه المجموعات ,مجموعه (G6) كانت بمثابة السيطرة السلبية، بينما تم تطعيم المجموعات الخمسة الاخرى. العمر والجرعة وطريق إعطاء مختلف اللقاحات تمت وفقا لتوجيهات الشركة المصنعه.

تم تلقيح المجموعة الأولى بلقاح نيوكاسل في اليوم السابع واعيد اللقاح في اليوم السابع عشر من العمر، تم تطعيم المجموعة ٢ مع لقاح الإنفلونزا في اليوم الاول واعيد اللقاح في اليوم الحادي والعشرين من العمر، وكانت هذه المجموعات بمثابة السيطرة الإيجابية. المجموعات ٣،٤،٥، تم تطعيمهمفي اليوم الاول واعيد التطعيم في اليوم العاشر من العمر واستخدمت BIVAC1 للمجموعة ٣، تم استخدام IBMA5 لتطعيم المجموعة ٤، في حين تم استخدام لقاح H120 للمجموعة ٥. بعد أربعة أيام من التطعيم، لوحظت العلامات السريريةلجميع المجموعات . وقد قتلت جميع الطيور التجريبية وتم فحصها باختبار حركة الاهداب بطريقه الفحص المباشر من خلال المجهر الضوئي واختبار صبغه الحبيبات الكاربونيه. اضهرتنتائج النشاط الهدبي للمجموعه الملقحه بلقاح نيوكاسل كان ٩٥% ,اما المجموعة الملقحة بلقاح الانفلونزا كان ٩٣%, في حين نتائج النشاط الهدبي للمجموعات الملقحهبالانواع الثلاث للالتهاب الشعب الهوائي كانت (21.33.20 %) على التوالي , بينما مجموعه السيطره (الغير ملقحه) كانت ١٠٠% .

REFERENCES

1. Mensah, G. A., and J. B. Brain. (1982) . Deposition and clearance of inhaled aerosol in the respiratory tract of chickens. J. Appl.Physiol. (53):1423–1428.

2. Bang, B. G., and B. M. Wenzel. (1985) . Nasal cavity and olfactory system. Form and Function in Birds. A. S. King and J. McLelland, ed. Academic Press, London .

3. Hayter, R. B., and E. L. Besch. (1974). Airborne-particle deposition in the respiratory tract of chickens. Poultry Sci. (53): 1507–1511.

4. Button, B. and R.C. Boucher.(2008). Role of mechanical stress inregulating airway surface hydration and mucus clearance rates. Respir. Physiol. Neurobiol. (163): 189-201.

5. Rubin, B.K. (2007). Designing clinical trials to evaluate mucus clearance therapy. Respir. Care (52): 1348-1361 .

6. Hogg, J.C., Chu, F., Utokaparrch, S., Woods, R., Elliot, W.M., Buzatu, L., Cherniack, R.M., Rogers, R.M.,Sciurba, F.C., Coxson,

H.O. and P.D. Pare(2004). The nature of smallairway obstruction in chronic obstruction pulmonary disease. N. Engl. J. Med. (350): 2645–2653.

7. Redding, G.J., Kishioka, C., Martinez, P. and Rubin, B.K. (2008). Physical and transport properties of sputum from children with idiopathic bronchiectasis. Chest. (134): 1129-1134 .

8. Dalhamn, T . and Rylandler, R'. (1971) : Reduction of Cigarette Smoke Ciliotoxicity by Certain .Amer . Rev . Resp . Dis . 103 :855.

9. Ferkol, T. and M. Leigh .(2006). Primary ciliary dyskinesia and newborn respiratory distress. Semin. Perinatol. (30), 335-340.

10. Wood, A.J. and R.G. Douglas.(2010). Pathogenesis and treatment of chronic rhinosinusitis. Postgrad.Med. J. (86): 359-364.

11. Lansley, A.B.(1993) . Mucociliary clearance and drug delivery via the respiratory tract. Adv. Drug Deliv.Rev. (11): 299-327.

12. Shirzad , M. R. ; K. Asisi and A. Mohammadi (2012).Efficacy of vaccination programs using two commercial live Infectious Bronchitis vaccines against field IRFIB32 strain. Bulgarian J. of Vet. Med. ,15, No (4) :260−272.

13. Heck& Tong (1977) . Protective action of PMO against cigarette smoke induced inhibition of mucociliary particle transport in the chicken trachea .1044.

14. Cook, J.K.; Orbell, S.J.; Woods, M.A. and Michael, B.(1999). Breadth of protection of the respiratory tract provided by different live-attenuated infectious bronchitis vaccines against challenge with infectious bronchitis viruses of heterologous serotypes. *Avian Pathology, (28):477-485.*

15. Mark W. Jackwood[B], Brian J. Jordan, Ha-Jung Roh[A], Deborah A. Hilt, and Susan M. Williams.(2015) . Evaluating Protection Against Infectious Bronchitis Virus by Clinical Signs, Ciliostasis, Challenge Virus Detection, and Histopathology, Avian Diseases 59(3):368-374.

16. Boroomand Z, Asasi K, Hosseinian S, Mohammadi A and Karimi M.(2011). Simple rapid detection for activity of cilia in virus infected chicken trachea. *Volume 15 (4): 404-413.*

17. Abdullah, S. M. (2010). Pathogenicity of some infectious bronchitis disease vaccines. M.Sc. Thesis – College of Veterinary Medicine – Baghdad University – Iraq .

18. Cavanagh D. (2007). Coronavirus avian infectious bronchitis virus. Vet Res 38:281–297.

19.Matthijs, M.G., J.H. van Eck, W.J. Landman, and J.A. Stegeman. (2003). Ability of Massachusetts-type infectious bronchitis virus to increase colibacillosis susceptibility in commercial broilers: a comparison between vaccine and virulent field virus. Avian Pathol. (32):473–481.

20. Wilson, R., Dowling, R.B. and A.D. Jackson, (1996) . The biology of bacterial colonization and invasion of the respiratory mucosa. . Eur. Respir. J. 9, 1523-1530.

21. Snyder, W. W. Marquardt, and S. K. Kadavil .(1982) , Ciliary Activity: A Criterion For Associating Resistance To Infectious Bronchitis Virus Infection With ELISA Antibody Titer . AVIAN DISEASES vol. 27 no. 2 .

22. Saif, Y. M.; Barnes, H. J.; Glisson, J. R.; Fadly, A. M.; McDougald, L. R. & Swayne, D. E. (2008).Diseases of Poultry. 12th Ed., Iowa State Univ. Press. Blackwell Publishing Company, Iowa, USA. PP. 117-129.

23. Cavanagh, D. (2003). Severe acute respiratory syndrome vaccine development: experiences of vaccination against avian infectious bronchitis coronavirus Avian Pathology. 32, 567-582 .