# LOSSES DUE TO INFECTIOUS BRONCHITIS VIRUS INFECTION IN LAYING AND BREEDING HENS

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## ABSTRACT

This study indicates that IBV infection of laying chickens is of high economical importance as it adversely affects their production potentials. IBV-infected hens lay eggs of inferior quality compared to the uninfected hens. There were significant differences in the daily egg production, egg weight, shell weight and in the internal quality of eggs laid by the IBV infected and uninfected hens. The IBV infection of developing embryos resulted into mortality, kidney lesions, stunting and curling and low hatchability compared to uninfected embryos. These effects were more severe on the SPF embryos than the embryos from the broiler breeder hens.

**Keywords:** Infectious bronchitis virus, layers, breeders, losses

## INTRODUCTION

Avian infectious bronchitis (IB) continues to be an economically important disease of chickens despite the use of live attenuated and inactivated IB virus (IBV) vaccines (Hassnain *et al.*, 1995). IBV causes pathology in respiratory and reproductive tracts and in the kidneys of chickens (Cunningham, 1975; Tyrrell *et al.*, 1978; Gelb *et al.*, 1981; Kinde *et al.*, 1991).

The IBV may cause mortality in young chicks, and drop in egg production; laying of soft-shell, mis-shapped, uneven size and poor internal quality eggs in laying chickens (Hamilton, 1978; Muneer *et al.*, 1988). The experimentally IBV-infected embryos are either curled and dwarfed in size or die soon after hatching (Loomis *et al.*, 1950; Muneer *et al.*, 1988).

Keeping in view the virulence of IBV for commercial laying and breeding chicken, the present work was designed to evaluate the effects of IBV (Arkansas-99 strain) on egg production and chicken embryo development and to correlate those effects with the economics of flock production.

## MATERIALS AND METHODS

### Experiment 1: Effects of IBV on laying hens

#### Experimental Birds

A total of 200, twenty-three week old white leg horn layers were wing banded and divided into two groups each consisting of 100 hens.

The daily egg production in pre-infection period was recorded at 65 percent in control group and 66.5 percent in group to be infected with IB Virus. The pre-and-post IBV infection serum samples from both the experimental groups were analyzed by haemagglutination inhibition test (Muneer *et al.*, 1988).

#### Administration of IBV Infection

The experimental hens were placed in two separate rooms having identical condition of temperature (70°F, 24 hourly), space (2 feet per bird) and light (16 hours per 24 hours). The birds were randomly housed 2 per cage in wire (30.45 cm) cages and provided feed and water *ad libitum*. Half of the birds in one group (both the birds from every other cage) were intra-ocularly inoculated with 0.05 ml Arkansas IBV suspension having a titre of $10^{5.5}$ $EID_{50}$ per ml. The uninoculated birds of the same group served as contact-infected birds; and the 100 hens in second group served as the uninoculated controls. Both the IBV-infected and uninfected control hens were examined at least twice daily upto 30 days post-infection for the evidence of any clinical signs. Serum samples from both the IBV infected and uninfected chickens were collected one day before administration of IBV challenge and on day of infection and every 7th day thereafter upto 28th day post-infection and evaluated using HI test (Muneer *et al.*, 1988; Wit *et al.*, 1997).

#### Evaluation of the Effects of Arkansas IBV Infection

Effects of IBV infection on eggs were judged on the basis of size, shape, weight, shell and internal quality.

Eggshell quality was evaluated on the basis of dry shell weight according to the procedure described by Hamilton (1978). Briefly, each individual egg was labeled, broken and the eggshell with shell membrane was preserved in an egg tray; eggshells were dried in an

oven at 105°C for 4 hours and weighed individually. The internal egg quality was determined on the basis of Haugh unit scores (Haugh, 1937). The data were statistically analyzed by the students T test at 5% level of probability.

**Experiment 2: Effects of IBV on developing chicken embryos**

To determine the effects of IBV on developing chicken embryos, IBV-Arkansas suspension in Hanks balanced salt solution, pH 7.2, containing 100 IU/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamicin and 50 IU/ml mycostatin was used. A total of 280 eggs from SPF flocks supplied by Salsbury Laboratories, Charles city Iowa, USA and 280 eggs from broiler breeder flock were incubated at 99.5°F for embryonic development. On day 9 post-incubation, 240 eggs each from the SPF layers and 240 broiler breeder hens indicating the presence of active and vigorous embryos with well-developed blood vessels were randomly divided into six groups (SPF embryos= A,B,C groups; Broiler breeder embryos= D,E,F groups) each group consisting of 80 embryos. Each embryo in groups A and D was inoculated with 0.1 ml of virus suspension containing $10^4$ $EID_{50}$ per ml via the allantoic sac, each embryo in groups B and E was inoculated via allantoic sac with diluent without virus and none of the embryos in groups C and F was inoculated with IBV suspension or diluent. The shell holes used for inoculation were sealed with wax and the eggs were re-incubated at 99.5°F. On days 2, 3, 4, 5, 6, 7, 8 and 9 of post-inoculation (PI), five embryos from each group were randomly selected, candled chilled at 4°C overnight and their allantoic fluids (AF) were harvested and pooled. The embryos were separated from the membranes and examined for the presence of pathological changes.

The embryos found dead within first 12 hours PI were discarded as accidental deaths but those dying later were chilled for observations. Parameters used to evaluate the effects of IBV on embryos included behaviour of embryos on candling, structural changes such as curling and dwarfing, motility, mortality and HA activity in AF and embryonic membranes. Samples consisting of trachea, lungs and kidneys from embryos showing pathological lesions and from uninoculated embryos were collected for histopathological observations. These samples were fixed in 10 percent neutral buffered formalin, embeded in paraffin, sectioned at 4 um and stained with haematoxylin-eosin for microscopic examination.

## RESULTS

The data obtained through this work indicates that IBV infection in laying and breeding flocks caused significant economical losses to farmers, as the effects of IBV on egg production and developing chick embryos were remarkable. Significant differences ($P<0.05$) were observed in the overall egg production, egg quality and hatching percentage between IBV infected and uninfected embryos. Data on post IBV infection clinical signs in layers, their immune response, daily egg production, egg size, egg weight, shell quality and interior egg quality are presented in Table-1. The effects of IBV on chick embryos at various age intervals are summarized in Table-3.

The HI test did not detect any significant GMT titre of antibodies in pre-IBV infection serum samples. Both, the IBV inoculated and contact infected birds had higher HI antibody titres than the uninoculated controls by day 7 post-infection, indicating that the IBV virus is transmissible through contact. The HI titres of IBV inoculated and contact infected birds increased upto 28 days post-infection (PI). The GMT HI titre of infected birds did rise from 64 on 7th day PI to a GMT HI titre of 1024 on 28th day PI, indicating that the infected birds had competent humoral immune system.

Only 5 of the infected birds indicated signs of illness. Those hens did not look very active and had slight oculo-nasal discharge from 4-8 days PI. Of the 50 IBV inoculated layers, one died on day 28 PI. Its necropsy indicated air-sacculitis, slight mucous in the trachea and bronchi, slight lung congestion and nephritis.

The egg production of IBV infected and uninfected birds differed markedly. On day 15 PI, daily production in IBV infected and uninfected hens was 62.08 and 77.08 percent, respectively. On day 30 PI, the egg production in IBV infected and uninfected layers was 61.25 and 85.25 percent, respectively. In addition, over 6% of the total eggs from the IBV infected flock were either soft-shelled or mis-shapen. The eggs from IBV infected hens were also smaller in size and weighed lesser. The average egg weight on day 20 post-infection in IBV infected birds was 59.6 grams compared to 64.4 grams of uninfected hens. The pre-infection shell weights were 6.1 g/egg for IBV infected layers and 6.3 g/egg for uninfected hens. By day 28 PI shell weight averaged 5.3 g/egg from IBV infected flock and 6.0 g/egg from the control layers. Approximately 0.75 percent of the eggs laid by infected hens had calcarious papule-like deposits on the outer-shell surface. No such effects on eggs from the uninfected layers were visible.

The IBV infected hens laid significantly more eggs that were graded as B and C (on the basis of Haugh Unit score) than the control hens ($P<0.05$). The IBV infected

eggs had a watery albumen compared to firm consistency of egg albumen from the uninfected layers.

**Effects of IBV on developing chicken embryos**

Ten days old developing chicken embryos were inoculated with IBV and incubated at 37°C upto 9 days PI (Table-3). On the day of inoculation, both the IBV inoculated and uninoculated embryos showed well developed blood vessels and vigorous movements on candling. By 24 hours PI, IBV inoculated embryos had surface congestion and were friable. No changes were detectable in the embryo size or visceral organs. Major effects of IBV inoculation were evident in the form of increased blood supply and sluggish embryo movements on candling, embryo curling, and dwarfism, poor feather germs development and decreased amount of amniotic fluid compared to uninfected embryos. Embryo mortality increased with the PI time. No significant gross or microscopic changes were observed in trachea, lungs or kidneys of IBV inoculated embryos upto 6 days PI. Marked inflammatory lesions in the kidneys were seen on days 7 and 8 PI. The tissue sections of lungs and kidneys of embryos on day7 PI indicated microscopic changes. Cellular debris was observed in para bronchii due to sloughing of epithelial cells, necrotic granulocytes and possibly aspirated amniotic membranes. The kidneys had calcified renal tubular casts especially in cortex and necrotic cells in renal tubular lumen. The effects of IBV-Ark on hatchability are summerized in table 2. In SPF embryos in groups A (IBV-inoculated), B (diluent-inoculated) and C (uniloculated) the hatchability percentages were 75,82.5, and 85, respectively. In embryos from commercial broiler breeder flock the hatchability percentages in IBV-inoculated, diluent inoculated and uninoculated groups were 80, 85, 87.5, respectively. The overall findings of this study were that the IBV-Ark infection in laying and breeding chickens adversely affected their production and hatchability potentials (Table 4), and thus caused economical losses to the farmers.

## DISCUSSION

IB corona-virus(IBV) may infect the laying chicken and cause poor egg production performance (Tyrrell *et al.*, 1978; Zhong *et al.*, 1995; Wang *et al.*, 1996), laying of inferior quality eggs (Muneer *et al.*, 1988) and dwarfism and stunting of embryos which increases with the serial passage of virus (Delaplane and Stuart., 1941; Cavanagh and Naqi, 1997).

The objective of this study was to determine the economical losses caused by IBV in the laying and breeding chickens.

The data obtained through this study suggests that IBV infected mature hens do not develop pronounced clinical signs following infection. However, those birds do seroconvert. Tests like ELISA, Immunodiffusion, virus neutralization and hemagglutination-inhibition are used for determination of antibodies against IBV (Gough and Alexander., 1978; Kind and Hopkins., 1983; Lashgari and Newman., 1984; Schultz *et al.*, 1992). However, in the present study HI test was used keeping in view the economy of reagents and easiness of its conductivity. The HA/HI tests were conducted with the phospholipase c treated antigen.

This study indicates that IBV infection has adverse effects on egg production potential of layers and on the quality of eggs.

Compared to the uninfected, IBV infected hens laid significantly less ($P<0.05$) number of eggs; and many of those eggs were soft shelled, mis-shapen and of poor internal quality. The eggs and shells from IBV infected birds were of lower weight and a few eggs had calcarious papule deposits on their surface. Over 0.25 per cent of the eggs from IBV infected hens had meat spots in their albumen. No such effects were observed on eggs obtained from the uninfected hens (Table 1). Observations of workers like Cook (1983); Muneer *et al.* (1988); Zhong *et al.* (1995); Wang *et al.* (1996); Cavanagh and Naqi (1997); support the findings of present investigation.

This study further suggests that IBV infection of developing chick embryos leads to detrimental effects on its morphology and hatchability. The major effects of IBV on SPF embryos inoculated with IBV included slow movements of the embryos on candling, generalized muscular congestion, curling and stunting, kidney lesions, and reduced hatchability in comparison with uninfected embryos or the embryos from broiler-breeder chickens (Table-3). The hatchability of un-infected, IBV infected SPF and IBV infected broiler breeder embryos were 87.5, 75.0 and 80.0 percent, respectively (Table 2). Similar findings on IBV have been reported by Cavanagh and Naqi (1997) and Delaplane and Stuart (1941). The SPF chickens had not been vaccinated for IBV during their rearing period. Non significant levels of HI antibody were detectable in the serum and egg yolk samples derived from SPF hens (GM HI serum titre = 8; and GM HI yolk titre = 12) at the time of initiation of embryo development. The absence of yolk antibody might have helped IBV to freely replicate and cause lesions in embryos. However, in contrast to SPF birds, the broiler breeder chickens had received IBV vaccination on days 3 and 20 (live H-120), at 8 weeks (live H-120), and 16 weeks (killed oil emulsion). This could be one reason of lesser susceptibility of broiler breeder embryos to IBV infection. The serum and egg yolk from those birds indicated a good HI antibody titres (serum titre 512-1024; egg yolk titre = 256-1024). However, these titres did not fully protect the embryos against heterologous IBV challenge (Table 3).

**Table 1: Major effects of IBV-infection in laying chickens**

| Type of Effects | | Uninfected layers | IBV-Infected Layers |
|---|---|---|---|
| Clinical disease | | NIL | Slight illness (oculonasal discharge)Loss of activity |
| Seroconversion | | | |
| * Pre infection | | 8 | 8 |
| * Post-infection | | | |
| | Day-07 | 16 | 64 |
| | Day-15 | 8 | 128 |
| | Day-21 | 16 | 512 |
| | Day-28 | 16 | 1024 |
| Egg Production %age | | | |
| * Pre-infection | | 76.00% | 77.0% |
| * Post-infection | | | |
| | Day-07 | 75.00% | 73.00% |
| | Day-15 | 77.08% | 62.08% |
| | Day-21 | 79.0% | 63.00% |
| | Day-30 | 85.25% | 61.25% |
| Egg Quality | | | |
| - | Misshappen, Soft-shell eggs | 0.5% | 6.00% |
| - | Average-egg weight day 28 P.I. | 64.45 gms | 59.6 gms |
| - | Average shell weight | 5.99 gms | .23 gms |
| - | Calcarious Papules | Nil | Present |
| - | Haugh Unit Scores | | |
| | Egg grade AA | 33.83% | 22.0% |
| | Egg grade A | 63.83% | 59.66% |
| | Egg grade B | 2.0% | 15.83% |
| - | Meat spots in albumen | Nil | Present |

P.I. = Post-Inoculation; GMT = Geometric mean titre

**Table-2: Effect of IBV-Ark on hatchability of chicks**

| Group | Treatment | Embryos inoculated | Hatched | Hatching %age |
|---|---|---|---|---|
| A[a] | IBV-inoculation | 40 | 30 | 75 |
| B[a] | Diluent inoculates | 40 | 33 | 82.5 |
| C[a] | Uninoculated control | 40 | 34 | 85 |
| D[b] | IBV-inoculated | 40 | 32 | 80 |
| E[b] | Diluent inoculated | 40 | 34 | 85 |
| F[b] | Uninoculated control | 40 | 35 | 87.25 |

a = Embryonating eggs from SPF hens; b = Embryonating eggs from broiler breeding hens

**Table 3: Effects of IBV on 9 days old developing chick embryo***

| Effect | Post-inoculation Period | Uninfected embryo** | SPF-IBV infected embryo | Broiler breeder IBV-infected embryo |
|---|---|---|---|---|
| Movements on candling | 24 hrs | Swift | Relatively slow | Swift |
| Muscular congestion | 24 hrs | Slight | Profuse | Slight |
| Mortality | 36 hrs.<br>48 hrs. | 5 percent<br>5 percent | 10 percent<br>10 percent | 7.5 percent<br>5.00 percent |
| Kidney lesions | 7-8 days | Absent | Present in 50 percent dead/alive | Very slight only in 25% dead embryos |
| Dwarfism stunting curling | 6 days | Absent | 7.5 percent | 2.5 percent |
| Hemagglutination titre in AF | 72 hrs. | Absent | Pooled titre HA=128 | Pooled titre HA=32 |
| AF yield per embryo | 72 hrs | 5-6 ml | 4-4.5ml | 6-8ml |
| Percent Hatchability | 12 days | 85-86 | 75 | 80 |

*Each of the SPF and broiler breeder embryo was inoculated with $10^4$ $EID_{50}$/ml of Arkansas strain of IBV;
**Number of observations of the fertile eggs incubated after candling and post-inoculation were 50 embryos each for the uninfected, SPF and broiler breeder chickens.
SPF = Specific Pathogen Free; AF = Allantoic Fluid; HA = Haemagglutination; EID = Embryo Infective Dose

**Table 4: Economical losses due to IBV infection in laying and breeding flocks**

| Parameter | Uninfected Birds | IBV-infected Birds | Difference | Losses in Rupees |
|---|---|---|---|---|
| **A) Production performance** | | | | |
| Percent Production on day 15 P.I. | 77.08 | 62.08 | 15 | Rs. 30.00[a] |
| Percent Production on day 30, P.I. | 85.25 | 61.25 | 24 | Rs. 48.00 |
| Percentage of Soft shell small size and misshapen eggs | 00.50 | 6.00 | 5.5 | Rs. 6.60[b] |
| Calcarious deposits | - | 0.75 | 0.75 | Rs. 2.00[c] |
| Egg grades<br>AA | 33.83 | 22.00 | 11.83 | Loss of acceptance by house wife |
| A | 63.83 | 59.66 | 4.17 | " |
| B | 2.00 | 15.83 | 13.83[d] | " |
| **B) Hatching performance** | | | | |
| Hatching Percentage<br>SPF-embryos | 85 | 75.00 | 10 | Rs. 160.00 |
| Broiler breeder embryos | 87.5 | 80 | 6.25 | Rs. 100.00 |
| Curling+dwarfing | Absent | Present | - | Loss of chick quality |
| Average chick weight (SPF eggs) | 36 grams | 33 grams | 3 grams | Loss of chick quality |
| Average chick weight (Broiler breeder eggs) | 38 grams | 32 grams | 6 grams | Loss of chick quality |

a = The table egg average price for the year 1998 in Pakistan was Rs. 2.04 per egg (Minimum price per egg= Rs. 1.54 was during the month of April; and the maximum price per egg= Rs. 2.72 was during the month of March,1998 (Source Pakistan Poultry Association); b = Small size and misshapen eggs sold at reduced rates (Rs. 12.00 per dozen); c =Calcarious deposits on eggs lead to very low price; d =B and C grade eggs not liked by the consumers especially the house wife; e =Presently average chick cost varies between Rs. 16.00 to Rs.24.00; f = Loss of quality of chicks: Curled and stunted B grade chicks are sold at around 50 per cent of the chick price and C grade chicks have to be culled.

Shane (1997) suggested the multiple inoculation of breeding flocks with live and killed IBV vaccines. Major factors contributing to the incidence of IB in chickens are multiple strains of the contagium, poor correlation of humoral neutralizing antibody titer to protection against reinfection with IBV, alteration in the antigenic characteristics and emergence of new and antigenically distinct IBV strains, non-availability of broad spectrum IBV vaccines and decline of immunity to resist reinfection/infection with IBV strains (Raggi and Lee, 1965; Cunningham, 1970; Hofstad, 1981; Cook, 1983). The findings of present work necessitate more work as protection afforded by the IBV vaccines was for homologous challenge only. Previous work suggests that IBV vaccines confer protection only against homologous challenge (Muneer *et al.*, 1988). Muneer and Khawaja (1994) conducted a sero-surveillance of breeding and laying flocks in Pakistan and reported significant levels of antibodies to Arkansas, Massachusetts (M-41), JMK, D-274 and D-1466 IBV types in the IBV-vaccinated and un-vaccinated flocks indicating that those viruses were circulating at poultry farms. Observations of Muneer and Khawaja (1994) call for more work on the efficacy of presently available IBV vaccines.

From the findings of present investigations, it can be concluded that IBV infection of laying and breeding flocks causes significant economical losses to the farmers (Table 4). Those losses are in the form of low egg production; laying of shell-less, soft-shelled, misshappen, small sized and inferior quality eggs. In addition, the eggs produced by the infected breeding flocks have lower hatchability and some of the newly hatched baby chicks die immediately after hatching. The egg production and hatchability losses can only be prevented by the use of efficient vaccines and vaccination programs; and monitoring for the effective immune response of vaccinates.

## REFERENCES


Cavanagh, D. and S.A. Naqi, 1997. Infectious Bronchitis In: Diseases of poultry. 10th Ed.. Edited by Calnek, B.W; Barnes, H.J; Beared, C.W;. McDougald, L.R; and Saif, Y.M. Iowa State University Press, Iowa, USA. pp: 511-526

Cook, J.K.A., 1983. Isolation of a new serotype of infectious bronchitis like virus from chicken in England. Vet. Rec., 112: 104-105.

Cunninghan, C.H., 1970. Review: Avian Infectious bronchitis. Adv. Vet. Sci. Cop. Med., 14: 105-148.

Cunningham, C.H., 1975. Avian infectious bronchitis: Characteristics of the virus and antigenic types. Am. J. Vet. Res., 36: 522-523.

Delaplane, J.P. and H.O. Stuart, 1941. The modification of infectious bronchitis virus of chickens as the result of propagation in embryonated chicken eggs. Rhode Island Agricultural experimental station Bulletin, 284:1-20.

Gelb, J. Jr., B.E. Perkins, J.K. Rosenberger and P.H. Allen, 1981. Serology and cross-protection studies with several infectious bronchitis virus isolates from Delmarva-reared broiler chickens. Avian Dis., 25: 655-666.

Gough, R.E. and D.J. Alexander, 1978. Comparison of serological tests for the measurement of the primary immune response to avian infectious bronchitis virus vaccine. Vet. Microbiol., 2:289-301.

Hamilton, R.M.G., 1978. Observations on the changes in the physical characteristics that influence egg shell quality in ten strains of white leghorns. Poult. Sci., 57:1192-1197.

Hassnain, H.H., A. Ibrahim, A. El-Attar and M.Z. Eldimerdash, 1995. Sero-epidemiological studies on some respiratory viruses in broilers. Assiut. Vet. Med. J., 33: 217-222.

Haugh, R.R., 1937. The Haugh unit for measuring the egg quality. US Poult. Magazine, 43: 33-41.

Hofstad, M.S., 1981. Cross-immunity in chickens using seven isolates of avian infectious bronchitis virus. Avian Dis., 25: 650-654.

Kinde, H., B.M. Doft, A.E. Castro, A.A. Bickford, J. Jr. Gelb and B. Reynolds, 1991. Viral pathogenesis of a nephrotropic infectious bronchitis virus isolated from gram commercial pullets. Avian Dis., 35: 415-21.

Kind, D.J; and S.R. Hopkins, 1983. Evaluation of the hemagglutination-inhibition test for measuring the response of chickens to avian infectious bronchitis virus vaccination. Avian Dis., 27: 100-112.

Lashgari, M.S. and J.A. Newman, 1984. Determination of the antigenic relationships within the Massachusetts (M41) type of infectious bronchitis virus using the hemagglutination-inhibition test. Avian Dis., 28: 444-452.

Loomis, L.N., C.H. Cunningham, M.L. Gray and F.J. Thorp, 1950. Pathology of the chicken embryo infected with infectious bronchitis virus. Am. J. Vet. Res., 11: 245-251.

Muneer, M.A., J.A. Newman, D.A. Halvorson, V. Sivanandan, K.V. Nagaraja and C.N. Coon, 1988. Efficacy of infectious bronchitis virus vaccines against heterologous challenge. Res. Vet. Sci., 45:2-27.

Muneer, M.A and K.N. Khawaja, 1994. Seropravelence of various infectious bronchitis virus strains in chickens.Final Technical Report. Pakistan Science Foundation, Islamabad.

Raggi, L.G. and G.G. Lee, 1965. Lack of correlation between infectivity. Serological response and challenge results in immunization with avian infectious bronchitis vaccine. J. Immunol., 94: 538-543.

Schultz, B., D. Cavanagh and G. Herrier, 1992. Neuraminidase treatment of avian infectious bronchitis coronavirus reveals a hemagglutinin activity that is dependent upon sialic acid-containing receptors on erythrocytes. Virology, 189:792-794.

Shane, S.M., 1997. Hand Book on Poultry Diseases. Printed by American Soybean Association. Vol. PO36, pp. 37 and 85.

Tyrrell, D.A.J., D.J. Alexander, J.D. Allmedia, C..H. Cunningham, B.C. Ensterday, D.J. Garwes, J.C. Hierhozler, R. Kapikian, M.R. MacNaughton and K. McIntosh, 1978. Coronaviridae: second Report. Intervirol. 10: 321-28.

Wang, C.H., M.C. Hsieh and P.C. Chang, 1996. Isolation, pathogenicity and H120 protection efficacy of infectious bronchitis virus isolated in Taiwan. Avian Dis., 40: 620-25.

Wit, J.J.de, D.r. Makkes, B. Kouwenhoven and J.H.M. Verheyden, 1997. Sensitivity and specificity of serological tests for detection of infectious bronchitis virus induced antibodies in broilers. Avian Pathol., 26:105-118.

Zhong Xu, W.U., Wer. Jianping, Z.X. WU and J.P. Wei, 1995. Influence of infectious bronchitis in young chickens on their late production. Chinese J. Vet. Med., 21:5-15.