Journal of Medicinal Plants Research Vol. 5(17), pp. 4304-4309, 9 September, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Effects of Chinese herbal formula *Maxing Shigan* powder on IgA secreting cells in chicken bronchus

Pengfei Liu[1], Wanyu Shi[1], Yantao Zhao[1] and Xiuhui Zhong[2]*

[1]College of Veterinary Medicine, Agricultural University of Hebei, Baoding 071001, P. R. China
[2]Institute of Traditional Chinese Veterinary Medicine, Agricultural University of Hebei, P. R. China

Accepted 14 June, 2011

In this paper the effects of a Chinese formula *Maxing Shigan* powder were investigated on IgA-secreting cells of chicken bronchus. The herbal powder was dissolved in water at three dosages, 0.5, 1 and 1.5g/L. Each dosage was given to 15-day-old chickens which were challenged with infectious bronchitis virus (IBV) by artificial infection for 3 days. Then the therapeutic effects were detected. The chickens were sacrificed at 18, 21, 24, 27 and 30 days old. The changes of IgA-secreting cells per unit area of bronchus were measured by immunohistochemistry. The IBV antibody titers in serum were determined by ELISA. The results of the therapeutic effects showed that *Maxing Shigan* powder can significantly reduce the mortality and enhance the recovery rate. Positive expression rate of bronchial IgA-secreting cells and higher IBV antibody titers were observed in the herbal treated groups. The results suggest that *Maxing Shigan* powder can protect the mucosa of IBV chickens, promote IgA secreting cells recruitment in the mucosa and secretion of the IBV antibody so as to lower the mortality and elevate the recovery rate of the IBV infected chickens.

Key words: *Maxing Shigan* powder, infectious bronchitis virus, IgA secreting cells, antibody.

INTRODUCTION

Infectious bronchitis is an acute, highly contagious disease caused by infectious bronchitis virus belonging to Coronavirus of Coronaviridae, which is one of the important diseases in intensive poultry industry (Cavanagh, 2007; Saif, 2005). In China, as in other countries, IB has occurred frequently in vaccinated and non-vaccinated flocks and has caused severe economic losses in recent years (Wang et al., 1997; Wu et al., 1998; Zhou et al., 2001; Ren et al., 2002; Chen et al., 2003; Liu and Kong, 2004; Ni et al., 2005; Yang et al., 2005). The appearance of antigenic variants of IBV causes a major problem in the poultry industry. Natural outbreaks of IBV are often the result of infections with strains that differ serologically from the vaccine strains (Cavanagh and Naqi, 2003; Cook, 1984; Wang et al., 1997; Ma et al., 2004). Mucous membrane in respiratory tract is the first barrier to invasion of exogenous pathogenic microorganisms of an animal. It removes or kills the pathogenic microorganisms directly with the help of mechanical barrier (tight junction, ciliary movement, mucous blanket etc.), immunologic barrier [Secretory immunoglobulinA (sIgA) etc.]. It is the important pathological component element in the outbreak of several diseases when the function of mucous membrane is damaged (Yang and Yang, 2006; Ding et al., 2006). sIgA is the major effecter molecule of mucosal immune system which mainly exists in tears, saliva, milk and the secretion of mucosal surfaces in respiratory tract, digestive tract and genitourinary tract. For the past years, the studies of sIgA have given rise to much interest. But now the research on interfering the barrier of mucous membrane in respiratory tract by relevant Chinese medicinal formulae is rarely reported. Abundant studies (Deng et al., 2005; Liu, 2004; He et al., 2007) have shown that many kinds of Chinese herbs have antiviral, anti-inflammatory and immunoenhancing properties. Consequently researches on such herbs on preventing and treating viral diseases have become a hotspot of traditional Chinese veterinary medicine at present. The prescriptions in previous reports are mostly used in

*Corresponding author. E-mail: zxh8078@163.com or zxh8078@hotmail.com. Tel/Fax: 86-312-2381158.

Abbreviations: sIgA, Secretory immunoglobulin A; **SPF,** specified pathogen-free; **APES,** aminopropyltriethoxysilane.

Table 1. Animal treatment in different groups.

Group	No. of birds	IBV challenge at 12 days of age	Herbal liquid (1g/L)
I	30	IBV	0.5
II	30	IBV	1
III	30	IBV	1.5
IV	30	IBV	——
V	30	——	——

Group I: Low dose of *Maxing shigan* powder group; Group II: Middle dose of *Maxing shigan* powder group; Group III High dose of *Maxing Shigan* powder group; Group IV: the model group (IBV challenge only); Group V: blank control group (no virus no herbal).

powder form as additives mixed with feedstuff only. This coarse herbal powder is inconvenient both for storage and use clinically. Therefore the classical formula *Maxing Shigan* pulvis is extracted by certain technology. The extracts are processed into water soluble fine powder; *Maxing Shigan* powder so as to be absorbed and utilized easily by chickens. In this study chickens were artificially challenged with IB virus. Immunohistochemistry was applied to determine the unit area of IgA-secreting cells in bronchus. The IBV antibody titers in serum were measured by ELISA. These methods were applied to investigate the enhanced protection effect of mucous membrane in IB chickens by *Maxing Shigan* powder. The study provides the theoretical basis to the therapeutics of IB with Chinese herbal medicine.

MATERIALS AND METHODS

Preparation of Maxing Shigan powder

The original formulae of *Maxing Shigan* pulvis consists of *Herba Ephedrae* 300 g, *almond* 300 g, *Gypsum Fibrosum* 1500 g and *Radix Glycytthizae* 300 g. *Gypsum Fibrosum* was broken into pieces and parceled before decoction for 0.5 h. The other herbs were added into the decoction liquid before pulverization. The herbs were decocted twice with the first 1.5 h and the second 1 h. The two parts of decoction liquid were pulled together and standing before filtration.

The filtrate was decompressed and concentrated to the density 1.16 (measured at 60℃). After cooling to room temperature, 95% alcohol was added so as to make the alcohol content to 50%. After standing for 12h, the supernatant was collected and decompressed for recycling the alcohol until the physic liquor is tasteless. After adding water to 1000 ml, the pH value was adjusted by 20% sodium hydroxide to 6.0~7.0.

After standing for 5 h and filtration, the filtrate was collected for decompressing and concentrating so as to make the density to 1.15 and then spray dried. At last adjuvant was added to make the weight to 1000 g. Each 100 g of *Maxing Shigan* powder was equivalent to 240 g of original crude herbs.

Virus strains and propagation

The stock virus was purchased from China Institute of Veterinary Drug Control. It was propagated, assayed and harvested from inoculated specified pathogen-free (SPF) chicken embryos. It was passaged for five generations. Each of the IBV-infected chickens was inoculated intratracheally with 0.2 ml preparation of the IBV strain $M_{41}E_4$, containing $10^{-6.32}$ egg lethal dose at 50% level (ELD_{50}).

Treatment of animals

One hundred and fifty one-day-old chickens were purchased from a commercial chicken farm in Shijiazhuang city, China, fed with standard feedstuff and tap water *ad libitum*. The birds were not vaccinated with any vaccines during the whole study period, and they were randomly allocated into five groups of 30 birds each.

The chickens were challenged with the virus harvested from inoculated specified pathogen-free (SPF) chicken embryos at 12 days of age. The challenged chickens (Groups I-IV) were housed in isolated hen cage. Birds in Group V received no virus challenge or herbal treatment. The room temperature was adjusted artificially. Low temperature environment was kept at 10-15℃ for 2-4 h daily. Other times it was kept at 18-20℃ together with sprinkling frequently in the hen cage to keep the indoor humidity at least 70%. Development of disease was observed closely after challenging. Clinical observation showed that chickens fell ill in succession 36 h after challenging, and then the whole flock was involved rapidly. The whole flock fell ill 48 h post challenge.

At this time point medication treatment in each medication group was started with *Maxing Shigan* powder by successive administration for 3 days. Experiment grouping and medication were listed in Table 1.

Preparation of reagents

SP-9002 Histostain™-Plus kits was purchased from Zhongshan Biotech Co., Ltd. (Beijing, China), consists of Bloking solution, 3% H_2O_2, Biotin-goat anti-mouse IgG, Streptavidin peroxidase (S-A/HRP) and Diaminobenzidine (DAB). Mouse anti-chicken IgA was obtained from Southern Biotech, Inc. (Birmingham, AL, USA). IBV antibody test kit was obtained from IDEXX Laboratories, Inc. (Westbrook, ME, USA).

Clinical therapeutic effect evaluation

Recovery rate

During the test period, a bird was judged cured if the clinical symptoms disappeared, spirit and ingestation has been restored. The number of curative chickens in each group divided by the whole number of chickens is the recovery rate.

Table 2. Test results of therapeutic effects.

Group	No. of birds	No. dead	Death rate (%)	No. cured	Recovery rate (%)	Total effective rate (%)
I	30	5	16.7B	19	63.3C	83.3C
II	30	2	6.7Cda	27	90A	93.3A
III	30	3	10BC	26	86.7AB	90AB
IV	30	12	40A	——	——	——
V	30	0	0Db	——	——	——

The different lowercase and capital letters showed significantly difference at 0.05 and 0.01 level, respectively. The same as follows.

Effective rate

During the test period, all the chickens are alive. Moreover, the symptom is reduced obviously with some chickens turn better in feces. General appearance, drinking amount and appetite also were monitoring signs as effective except complete healed. The number of living chickens in each group takes the percentage of the number of text chickens is the effective power.

Death rate

We judge chicken for dead if the it was found with IBV characteristic pathological changes only, exclusive of the other dead ones; the number of dead chickens in each group takes the percentage of the number of text chickens is the death rate.

Sample collection

At five time points, 18, 21, 24, 27, 30 day-old, 6 randomly selected birds per group were sacrificed, blood samples were taken by cardiopuncture and serum was collected for detecting IBV antibody titers. The sample of the bronchus was removed and fixed in Bouin's solution to detect the sIgA secreting cells.

Immunohistochemical examination for IgA secreting cells in bronchus

The fixed samples were embedded in paraffin. 5 μm thick sections of bronchus were serially cut and mounted on aminopropyltriethoxysilane (APES) coated slides. Twenty sections were prepared for each bronchus sample. The slides were treated with xylene twice, 7 min for each step. After dehydrating in graded ethanols, the endogenous peroxidase activity was neutralized by 3% H_2O_2 for 30 min. Immunohistochemistry staining was carried out with mouse anti-chicken IgA (1:200) according to the manufacturer's instructions. All incubations were performed in a moisture chamber. Control staining was carried out simultaneously in which the first antibody was replaced by PBS. No specific staining was found in the control.

ELISA examination for IBV antibody titers in serum

The IBV antibody titers in serum were determined using ELISA kit according to the manufacturer's instructions.

Histological observation

The sections were observed under Olympus BH-2 microscope, 4 and 10 times eyeshot were chosen per section, 10 sections per group. The data of positive cells were calculated by areas. The areas of IgA secreting cells in five different microscope fields were counted by Scion Image software for the statistical analysis of the data.

Data statistics

The data were assessed by one-way analysis of variance, using the SPSS 13.0 software, $p < 0.05$ was considered statistically significant.

RESULTS

Clinical therapeutic results

After challenged with IBV, death rate of chickens in the model group was significantly higher than that in the blank control group ($P < 0.01$). Death of birds was observed 60 h post challenge. Death rate of all the three herbal treated groups was significantly lower than the model group ($P < 0.01$). Among the three herbal treated groups, the recovery rates of Groups II and III were significantly higher than Group I ($P < 0.01$). Three days medication in Groups II and III showed satisfactory results, treatment effectiveness of Groups II and III being the most effective (Table 2).

The distribution characters and changes of IgA secreting cells

The results of immunohistostaining showed that IgA secreting cells were distributed abundantly in lamina propria of bronchus, round or oval in shape. The cytoplasm showed strong positive reaction whose color was dark brown. The nucleus showed negative reaction without coloring. Structures of trachea were undamaged in the blank control group (A and B) and the cilium was faintly visible (B). In model group (C and D), the structure of trachea was confused, the cilium was invisible. The bound among mucous gland lamina propria and submucosa was unobvious, and the number of IgA

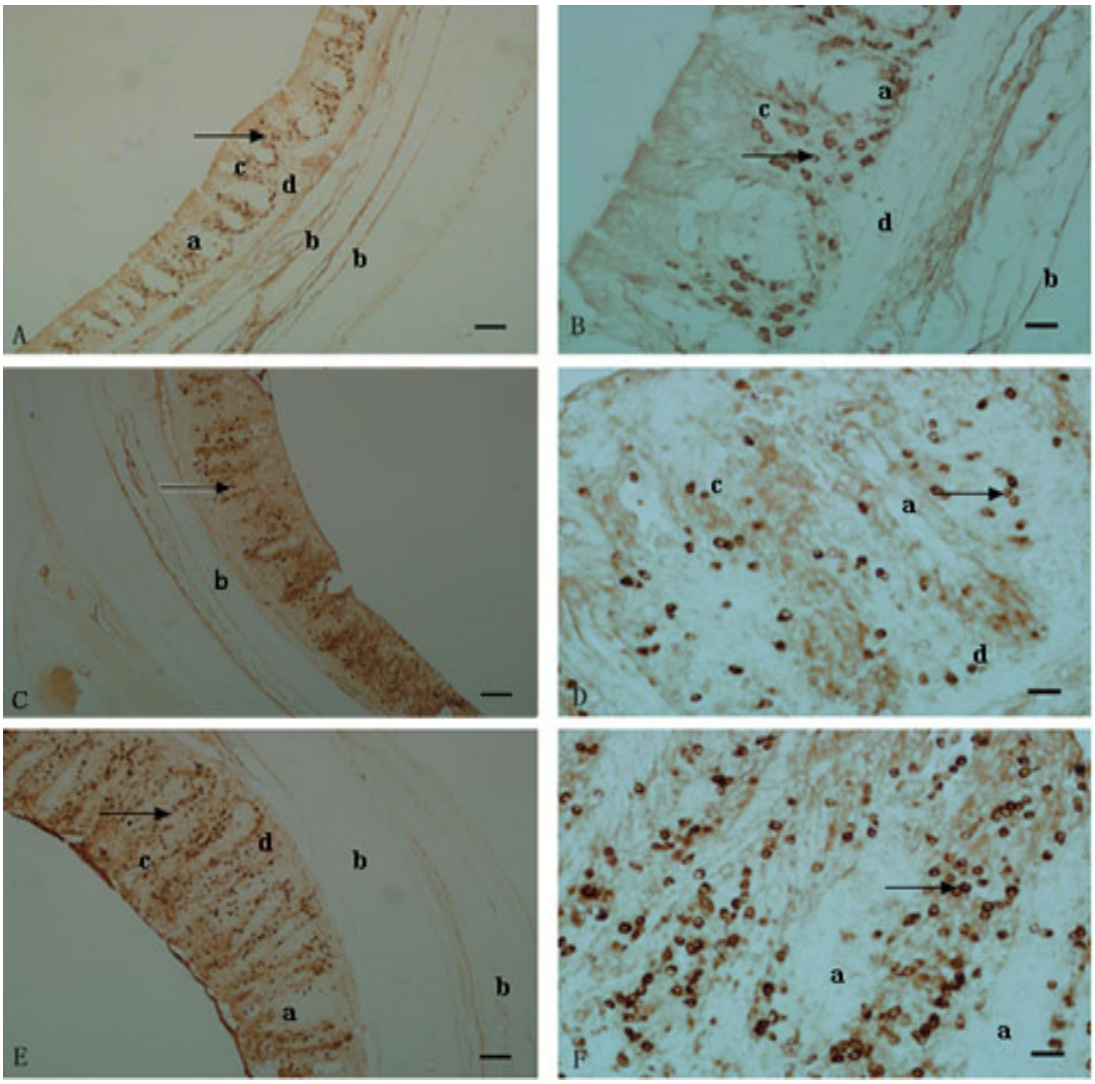

Figure 1. A. IgA secreting cells in blank control group, mucous gland. The bound between lamina propria and submucosa is obviously. Scale bar: 100μ m; B. IgA secreting cells in blank control group the secreting cells are well-distributed in lamina propria. Scale bar: 25 μm; C. IgA secreting cells in model group.The bound is in chaos, the stratum mucosum of the bronchus is getting thinner and easy to fall off. Scale bar: 100 μm; D. IgA secreting cells in the model group, the distribution of the secreting cells is confused and disorderly. Scale bar: 25 μm; E. IgA secreting cells in middle dose group, mucosa becomes thicker, the structures start to distinct. Scale bar: 100 μm; F. IgA secreting cells in middle dose group, the number of the secreting cells is significantly increased. The IgA secreting cells were pointed by the arrows. Scale bar: 25 μm. In all the above figures, a: mucous gland; b: tracheal cartilage; c: lamina propria; d: submucosa.

secreting cells increased significantly than the blank control group. In the group of middle dose (E and F), the structures were more distinct than the model group. The bound among lamina propria and submucosa was getting obvious.

The number of IgA secreting cells was the largest compared with the previous groups (Figure 1). The data analysis results displayed that, under normal circumstances, positive expression rate of bronchial IgA-secreting cells maintained a certain level. At 3 days post-infection (18-day-old), the IgA-secreting cells in groups challenged with virus were increasing significantly, and showed difference compared with the blank control group ($p<0.05$). At 6 days post-infection (21-day-old), positive expression rate in groups I, II and III showed significant differences compared with the model group ($p<0.01$ in Groups II and III). The positive expression rate of challenging groups continued to rise onwards, but the increasing amplitude of groups I and IV was not as high as in groups II and III, which was still higher than other three groups (Groups I, IV, V) significantly ($p<0.01$). Moreover, at 30-day-old, groups II and III still maintained

Table 3. The influence of *Maxing Shigan* powder on the IgA secreting cells.

Group	n	18-day-old	21-day-old	24-day-old	27-day-old	30-day-old
I	6	5.48 ± 0.52^{Aa}	6.51 ± 0.25^{ABb}	8.93 ± 0.16^{ABc}	8.51 ± 0.17^{Bc}	8.61 ± 0.19^{B}
II	6	5.98 ± 0.24^{A}	8.71 ± 0.42^{A}	11.03 ± 0.55^{Aa}	11.55 ± 0.31^{Aa}	11.92 ± 0.17^{A}
III	6	5.66 ± 0.45^{A}	7.63 ± 0.55^{Aa}	10.32 ± 0.44^{Ab}	10.95 ± 0.47^{Ab}	11.25 ± 0.52^{A}
IV	6	5.13 ± 0.25^{Ab}	5.92 ± 0.16^{Bc}	6.71 ± 0.40^{Bd}	6.90 ± 0.34^{Bd}	6.95 ± 0.31^{C}
V	6	3.30 ± 0.42^{B}	3.37 ± 0.39^{C}	3.14 ± 0.22^{C}	3.41 ± 0.18^{C}	3.28 ± 0.12^{D}

Table 4. IBV antibody titers in serum.

Group	n	18-day-old	21-day-old	24-day-old	27-day-old	30-day-old
I	6	172.96 ± 19.77^{Bc}	340.78 ± 17.32^{Bc}	506.85 ± 104.57^{Ba}	575.96 ± 25.72^{Ba}	475.96 ± 25.72^{Cd}
II	6	489.96 ± 53.23^{Aa}	615.22 ± 74.64^{Aa}	952.40 ± 122.28^{A}	1172.80 ± 85.10^{A}	864.24 ± 1341.64^{A}
III	6	403.70 ± 54.44^{Ab}	517.04 ± 28.39^{Ab}	907.60 ± 68.38^{A}	1032.17 ± 54.95^{A}	875.40 ± 55.13^{ABa}
IV	6	165.03 ± 68.61^{Bc}	317.32 ± 51.70^{Bd}	445.47 ± 30.06^{Bb}	526.75 ± 85.67^{Bb}	617.46 ± 101.61^{BCb}
V	6	77.65 ± 34.74^{Bd}	69.23 ± 39.40^{C}	72.10 ± 35.86^{C}	106.28 ± 86.85^{C}	173.22 ± 34.38^{D}

upward trend (Table 3).

Effect of maxing Shigan powder on IBV antibody

The result of antibody determination demonstrated that antibody titers of Group V were negative. Antibody titers in Group IV were increased significantly compared with Group V. But it demonstrated positive only when the birds were soon after resistance (24-day-old). After administered with *Maxing Shigan* powder, antibody titers in serum increased significantly in groups Ⅱ and Ⅲ. Furthermore, in each age level, they showed significant differences with Group Ⅳ ($P<0.01$). Among the drug treated groups, the middle and high dose groups showed higher titers than the low-dose group. In addition, there were significant differences at days 21, 24, 27 and 30 ($P<0.01$) (Table 4).

DISCUSSION

The surface of mucous membrane is the chief invaded site of foreign antigenic properties such as bacteria, virus and parasites. Therefore, the mucosal immune system constitutes the first immunologic barrier for animal organism to resist the intrusion by pathogenic microorganism (Yang, 2001). There is a powerful mucosal immune system in animal organism including both humoral and cellular immune function, and humoral factor plays the main parts. IgA secreted by IgA-secreting cells (sIgA) is the major effecter of humoral immunity. It prevents bacteria and virus invading through the surface of mucous membrane so as to prevent the formation of colony. Moreover, it agglutinates bacteria into macrobead, make the removing of bacterial particles by mucous membrane more conveniently. Co-existence with complement and lysozyme, IgA combats infection by dissolve bacteria. In addition, sIgA regulates phagocytosis and cytotoxicity mediated by antibody-dependant cell mediates (Tagliabue et al., 1983). The first door IBV invaded into was mucous membrane system of respiratory tract where immune response was generated. Under pathological conditions, the mucous membrane barrier was markedly destroyed, the ability of immunoreaction then became lower, pathogenic microorganisms and their toxic products would invade into the body, producing harmful effects. This can be seen in the present study. The results of immunohistostaining showed that in the model group there was a short time elevation in the quantity of IgA secreting cells after virus challenge, after that period the IgA secreting cells remained at lower level. Without adequate antibody production for self-protection, thereby virus invaded and the birds were severely harmed.

The mortality was significantly higher than the three herbal treated groups and the blank control group. In this research, stimulus in respiratory tract by IBV made the quantity of IgA secreting cells stayed at higher levels than the blank control group significantly. This phenomenon was more obvious in the middle and high dose groups which showed significantly more IgA secreting cells in the later stage of the study (Table 3). The results indicated that during the initial stage of virus challenge ,the surface of mucous membrane of the herbal treated birds stimulated by virus antigen started to secret specific antibody immediately for immunoprotection, considerable antibody titer still remained at high levels even in the later stage of the research. Clinically the herbal treated groups exhibited significant raise in recovery rate and effective rate. Clinical therapeutic results showed that mortality in

herbal treated groups decreased significantly and recovery rate and effective rate increased significantly (Table 2). This possibly is relevant with the protective effects of respiratory tract by the Chinese herbal medicine.

Antigenic stimulation would transform B lymphocytes into phlogocytes which secrets antibody with or without the assistance of T helper cells. The antibody level was an important index for humoral immunity (Qiu et al., 2007). In this research antibody levels in serum were monitored by ELISA after virus challenge and herbal treatment in order to investigate the beneficial effects of the Chinese herbal medicine on production and maintenance of antibody levels. The result showed that *Maxing Shigan* powder promoted the secretion of antibody, resulting in effective protection of the birds. The antibody titers were especially higher in groups of middle and high dosages (Table 4) which coincidence with the results of clinical therapeutic effects. The study showed that *Maxing Shigan* herbal powder has the ability to strengthen the immune response significantly, thereby cut down mortality of the disease.

Earlier studies have found that glycyrrhizic acid has the ability of non-specific immunoregulation by enhancing cellular immunity, reinforcing phagocytosis of macrophage, eliminating the inhibitory activity of suppressor macrophage, even more reinforcing the helper T cells by increasing CD4+ T cells and decreasing CD8+ T cells. Glycyrrhizic acid also regulates the formation and secretion of many kinds of cytokines, promoting IL-2, IFN-γ produced by lymphocyte and restraining the generation of IL-4 or IL-10 (Yoshikawa et al., 1997; Xu and Zou, 2005). How the whole herbal formula exerts the immunomodulation needs more concentrated studies. In a word, *Maxing Shigan* powder has the ability to lower the mortality of IBV infected chickens, to strengthen their immune function of mucous membrane especially secreting antibody at high levels at the stage of rehabilitation. Therefore the ability of defending the invading by strong viruses was elevated. Certain reference data was supplied that the research and application of the treatment of IB by the traditional Chinese medicine.

ACKNOWLEDGEMENT

This study was financially supported by the Ministry of Science and Technology of China (No. 2011BAD34B02).

REFERENCES

Cavanagh D, Naqi S (2003). Infectious bronchitis. In: Saif YM, Barnes HJ, Glisson JR, Fadly, AM, McDougald LR, Swayne DE (eds). Diseases of poultry. Iowa State Press, Ames, pp. 101-119.

Cavanagh D (2007). Coronavirus avian infectious bronchitis virus. Vet. Res., 38(2): 281-297.

Cook JKA. (1984). The classification of new serotypes of infectious bronchitis virus isolated from poultry flocks in Britain 1981 and 1983. Avian Pathol., 13: 733-741.

Deng JL, Yu SM, Zu ZC, Liao YH, Chen JJ, Zhuo LT (2005). Experimental Treatment and Prevention of Nephrapathogenic Infectious Bronchitis in Broiler Chickens with Chinese Herbal Medicinal Prescription. Chin. J. Vet. Sci., 25(2): 206-207.

Ding JZ, Zhang LT, Qiu XF, Gong Q, Zhu HL, Wang C, Zhang Y, Tan LH (2006). Effects of External-Dryness on Ciliary Movement of Tracheal Epithelium and Secretion of the Respiratory tract. J. Trad. Chin. Med., 47(8): 607-609.

He Y, Chen ZW, Liu WF (2007). Clinical Effect of Chinese Herbal Compound on Avian Infectious Bronchitis. Heilongjiang Anim. Sci. Vet. Med., 2: 74-75.

Liu SG, Kong XG (2004). A new genotype of nephropathogenic infectious bronchitis virus circulating in vaccinated and nonvaccinated flocks in China. Avian Pathol., 33: 321-327.

Liu ZX (2004). Clinical Observation of Chinese Herbal Medicine on Avian Infectious Bronchitis. Chin. J. Vet. Med., 40(10): 34-35.

Ma FL, Fu X.L, Chen GZ, Jia SY, Chen RM, Liu WS, Xuan H (2004). The isolation, identification and experimental vaccination of avian infectious bronchitis virus causing proventriculitis. Chin. J. Vet. Sci., 24: 225–227.

Ni B, Zhang YM, Sun P, Wang JY, Mu Y, Zhang YG, Wei ZF, Xu H (2005). Isolation and identification of kidney strain of avian infectious bronchitis virus. Chin. J. Vet. Sci. Technol., 35: 377-380.

Qiu Y, Hu YL, Cui BA, Zhang HY, Wang YG (2007). Effects of Achyranthes Bidentata Polysacchride on Immune Efficacy of Vaccine in Chickens. Chinese J. Anim. Vet. Sci., 38(7): 723-727.

Ren T, Liao M, Luo KJ, Cao WS, Xin CA (2002). The isolation and characterization of avian infectious bronchitis virus isolates in China. Acta Vet. Zoo. Tech. Sin., 33: 362-365.

Saif YM (2003). Diseases of poultry. 11th ed. Ames, IA: Iowa State University Press, pp. 108-130.

Tagliabue A, Nencioni L, Villa L, Keren DF, Lowell GH, Boraschi D (1983). Antibody-dependent cell-mediated antibacterial activity of intestinal lymphocytes with secretory IgA. Nature, 306(5939): 184-186.

Wang HN, Wu QZ, Huang Y, Liu P (1997). Isolation and identification of infectious bronchitis virus from chickens in Sichuan. China. Avian Dis., 41: 279-282.

Wu ZQ, Yang QW, Fu C, Zhao XY, Ignjatovic J (1998). Antigenic and immunogenic characterization of infectious bronchitis virus strains isolated in China between 1986 and 1995. Avian Pathol., 27: 578-585.

Xu QX, Zou M (2005). Outlines of Pharmacological Action of Glycyrrhizae. J. Practical Trad. Chin. Med., 21(7): 450-451.

Yang JH, Diao YX, Yu SY, Liu X, Jiang SJ, Liu YZ, Chen QP (2005). Isolation and sequence analysis of S1 gene of avian infectious bronchitis virus a field strain. Virol. Sin., 20: 283-287 .

Yang PH, Yang LF (2006). Recent Advances of Defence Mechanisms Against Influenza Virus Infection in the Respiratory Tract. Int. J. Immunol., 29(5): 305-309.

Yang SJ (2001). Mucosal Immunity. Foreign Medical Sciences Section of Immunol., 24(6): 296-299.

Yoshikawa M, Matsui Y, Kawamoto H, Umemoto N, Oku K, Koizumi M, Yamao J, Kuiyama S, Nakano H, Ishizaka S, Fukui H (1997). Effects of glycyrrhizin on immune-mediated cytotoxicity. J. Gasterol. Hepatol., 12(3): 243-248.