Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Colonization Pattern of *Bordetella avium* in Experimental Infection of Chicken

Ping-Ping Yang, Xue Zhao, Jing-Jing Liu, Yuan-Li Hao, Guan-Hua Liu, Xiao-Hua He and Rui-Liang Zhu*

College of Animal Science and Veterinary Medicine, Shandong Agricultural University, Tai'an, Shandong 271000, PR China
*Corresponding author: zhurl@sdau.edu.cn

ARTICLE HISTORY (12-357)

Received: September 22, 2012
Revised: April 05, 2013
Accepted: October 18, 2013

Key words:
Bordetella avium
Detection
Colonization
SPF chicken
Indirect immunoenzyme histochemistry

ABSTRACT

To analyze pathogenicity changes of new isolate of *Bordetella avium* LL09 from chick embryos, 120 1-day-old specific pathogen free (SPF) chickens were intranasally inoculated with broth cultures of isolate LL09. Its colonization pattern in different chicken tissues was studied by bacterial isolation and indirect immunoenzyme histochemistry. Results showed that the bacteria were isolated from tracheas and lungs at 1 h post-infection. Afterwards, they colonized livers, hearts and spleens at 120 h and then infected kidneys at 168 h. The peak infection appeared on 21 d post-infection. They persisted in these organs and caused injuries up to 42 d. With growth of chickens, *Bordetella avium* began to be gradually cleared away from livers, hearts and spleens, except that it could still be detected in tracheas, lungs and kidneys until 56 d post-infection. It demonstrated that lungs and kidneys would possible be colonized for a long time by *B. avium* in addition to the tracheas.

To Cite This Article: Yang PP, X Zhao, JJ Liu, YL Hao, GH Liu, XH He and RL Zhu, 2014. Colonization pattern of *Bordetella avium* in experimental infection of chicken. Pak Vet J, 34(2): 193-196.

INTRODUCTION

Bordetella avium (formerly called *Alcaligenes faecalis* type I) was first isolated from young turkeys in 1967, and was officially named *B. avium* in 1984. It's mostly regarded as pathogenic in commercially grown turkeys (Sacco *et al*., 2000). However, it's also found in numerous other species of wild and domestic birds (Raffel *et al*., 2002; Odugbo *et al*., 2006; Vichi *et al*., 2011; Liang *et al*., 2013). Human cases of respiratory disease associated with *B. avium* were only recently reported in patients with cystic fibrosis or chronic obstructive pulmonary disease (Spilker *et al*., 2008; Harrington *et al*., 2009).

In China, Ruiliang Zhu was the first to isolate *B. avium* from diseased chickens with feature of ocular discharge in 1991 (Zhu *et al*., 1991; Yang *et al*., 2012). However, in recent years our research group constantly isolated *B. avium* strains from embryos in some breeder hatcheries with reduced hatchability and increased weak chicks (Liu *et al*., 2006). The chicken is one of the most economically important animals in China. Avian bordetellosis has caused significant economic concern to producers of chickens. The insights gained from a systematic study of its pathogenicity to chickens may be useful in understanding the pathogenesis of *B. avium*.

Scholars in different countries have focused for years on the harm of *B. avium* to tracheas of turkeys (Temple *et al*., 1998; Kirby *et al*., 2001; Temple *et al*., 2010; Beach *et al*., 2012). It is extremely considered that pathogenicity of *B. avium* is associated with the damages of its virulence factors to tracheas (Murphy *et al*., 2002; Shelton *et al*., 2002; Spears *et al*., 2003; Kirby *et al*., 2004; Loker *et al*., 2011). Poosala Suresh localized and monitored *B. avium* in trachea sections of infected turkeys with indirect immunofluorescence in 1993 (Suresh, 1993). It has also been determined that *B. avium* colonizes lungs of infected turkeys in addition to the upper respiratory tract. However, no reports were found about its colonization in visceral organs to date. Furthermore, reports about its pathogenicity in poultry other than turkeys are extremely scarce.

As the first step in analyzing pathogenicity of *B. avium* isolate from chick embryos, its colonization pattern in different viscera of specific pathogen free (SPF) chickens was studied by bacterial isolation and indirect immunoenzyme histochemistry under the condition of artificial infection.

MATERIALS AND METHODS

Bacterial isolate: *B. avium* LL09 was isolated from 18-day-old chick embryos of one large Highland Brown breeder hatchery. Genebank accession number for sequence of its partial 23S rRNA gene is HM545299.

Chickens: Two hundred and forty 1-day-old SPF chickens (Saishi, China) were raised in biosecurity isolators. No bacteria were isolated from them. Six immunosuppressive viruses (including REV, ALV-J, CIAV, MDV, VAV and IBDV) were not found by PCR and RT-PCR, which were detected as described previously (Nolte and Caliendo, 2003; Register and Yersin, 2005). Blood samples for serological testing were taken from the jugular vein using sterile technique. No maternal antibodies to *B. avium* in serum were detected by plate-agglutination test.

Establishment of indirect immunoenzyme histochemistry: As described previously (Probst *et al*., 2005; Ünver and Saraydın, 2012), tissues obtained at necropsy were fixed in 10% buffered formalin. Then sections (4 μm) were prepared for detection. Having been optimized repeatedly, the work condition of indirect immunoenzyme histochemistry was determined as follows: Tissue sections were incubated sequentially with 1% (wt/vol) BSA for 1 h, with mouse anti-*B. avium* polyclonal antibodies obtained by immunizing mice with outer membrane proteins of *B. avium* which were extracted as described (Munir *et al*., 2010) (ELISA titer was up to 1:1280; used at a 1:50 dilution) at 37°C for 1.5 h, and with goat anti-mouse IgG conjugated to horseradish peroxidase (Solarbio, China; used at a 1:40 dilution as directed by the manufacturer) at 37°C for 30 min in a humid chamber. Finally sections were colored using a DAB staining kit (Tiangen, China) in dark place for 5 min, and then counterstained with hematoxylin for final microscopic examination.

Experimental infection: A total of 240 1-day-old SPF chickens were randomly divided into 2 groups. One hundred and twenty chickens in challenge group were intranasally infected by 10^7 cfu of *B. avium* isolate. The other 120 chickens were served as negative control, and they were inoculated with sterile brain heart infusion (BHI) broth (Difco, USA) which was used to incubate and dilute *B. avium*. Two groups of chickens were raised separately in different biosecurity isolators. They fed and drank freely.

Within 1 to 12 h post-infection, 3 chickens were euthanized and taken for autopsy per hour. Tracheas and lungs were obtained under axenic conditions, and were mixed with 1 ml sterile PBS (pH7.4) after ground with homogenizer. These suspensions were serially diluted 10-fold, and 0.1 ml of each dilution was spread on MacConkey agar plates. These plates were incubated for 24 h, and colonies were counted as described previously (Li *et al*., 2011). During 1 to 14 d post-infection, 3 chickens were euthanized per day. Blood samples for bacterial isolation were taken from wing vein using sterile technique. Six kinds of organs including tracheas, lungs, livers, hearts, spleens and kidneys were obtained at necropsy under axenic conditions. Bacteria were isolated from half of these organs at first. The other half of the organs were fixed in 10% buffered formalin. Sections (4μm) were prepared and stained as described above for final microscopic examination. In addition, 6 chickens were autopsied per week for infection rate calculation during 1 to 8 w post-infection and examined as 1 to 14 d post-infection. At the same time, antibody response to *B. avium* at different time points was examined by plate-agglutination test as described previously (Guo *et al*., 2010). Chickens in the control group were sampled and examined as those in challenge group.

RESULTS

Colonization pattern of *B.avium* in infected chickens: Some of infected chickens showed obvious clinical signs of respiratory disease such as coryza after intranasal infection. No bacteria were detected in blood post-infection. *B. avium* was detected in tracheas and lungs at 1 h post-infection with a bacterium quantity of 60 cfu in lungs. As time prolonged, *B. avium* continually increased and reached 2.8×10^3 cfu in lungs at 12 h post-infection. Afterwards, other organs such as livers, hearts and spleens were infected on 5 d and then kidneys were infected on 7 d post-infection.

In order to get significant insight into the knowledge of germ-carrying time and organ-addiction, number of *B. avium* in different organs was individually investigated at different times and results were represented in Fig. 1. Number of *B. avium* in viscera was the most on 21 d post-infection.

Infected proportions of different tissues at different times were listed in Table 1. Both tracheas and lungs had an infection rate of 100% in the initial 1 to 5 w. However, visceral infection didn't occur to all chickens, with a proportion of 1/3-1/2. After 28 d post-infection, *B. avium* appeared to be cleared from these tissues, except that colonization of tracheas, lungs and kidneys persisted until 56 d post-infection.

Table 1: Infected (positive) proportions (%) of different tissues at different times post-infection.

Time (days)	Trachea	Lung	Liver	Heart	Spleen	Kidney
1	100	100	0	0	0	0
7	100	100	33.3	33.3	33.3	16.7
14	100	100	50	50	50	50
21	100	100	50	50	50	50
28	100	100	33.3	33.3	33.3	33.3
35	100	100	16.7	16.7	16.7	50
42	100	66.7	0	0	0	33.3
49	100	50	0	0	0	33.3
56	100	16.7	0	0	0	33.3

Antibody response: Antibody response to *B. avium* detected by plate-agglutination test at different time points could be seen from Fig. 2. Antibody level was in a growth trend post-infection, especially, linear upward in the first 3-4 w post-infection. Antibody level reached a high level, more than 2^4, after 3 w. It showed a slow upward trend after 4w post-infection.

Colonization of *B. avium* in different organs: It could be seen from Fig. 3 that *B. avium* colonized extensively in tracheas, lungs, livers, hearts, spleens and kidneys. In tracheas, *B. avium* adhered to the ciliated tracheal cells in the beginning and resulted in cells shedding as shown by empty arrow. Then they colonized inner cells of the trachea as shown by solid arrows (Fig. 3-A). In lungs, *B. avium* primarily picked alveolar walls as the sites for colonization (solid arrows), and resulted in hemorrhage (empty arrow) as shown in Fig. 3-B. They colonized

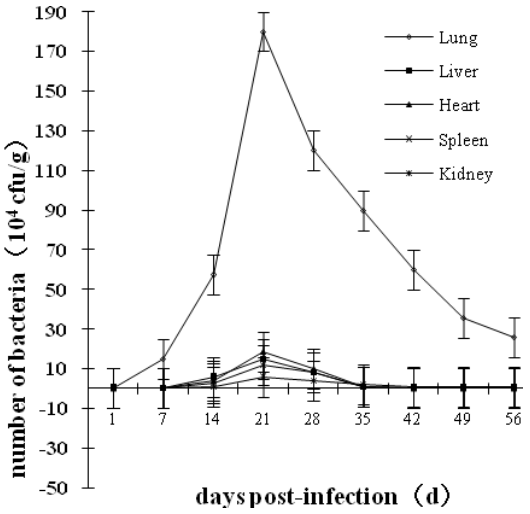

Fig. 1: Bacterial counts (cfu/g) from various organs in chickens after B. avium LL09 intranasal challenge. Data points represent mean value from 6 survives. Error bars are ± standard deviation.

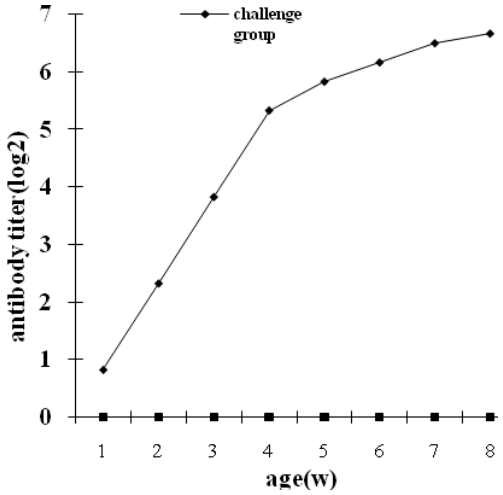

Fig. 2: Antibody response to *B. avium* detected by plate-agglutination test at different time points. Antibody level was in a growth trend post-infection.

densely in intercellular space and cytoplasm in livers and spleens (Fig. 3-C and Fig. 3-E). In hearts, they were mainly distributed along the myocardial cells (Fig. 3-D). In kidneys, they colonized in tubular epithelial cells and resulted in disintegration of them as shown by arrows in Fig. 3-F.

DISCUSSION

It's known to all that *B. avium* can colonize the upper respiratory tract, and cause coryza, rhinitis and ophthalmia, etc. in turkeys or other poultries (Raffel *et al*., 2002; Odugbo *et al*., 2006). For a long time, studies about pathogenicity of *B. avium* have been focused on its damage to the respiratory tract (Temple *et al*., 1998; Sebaihia *et al*., 2006; Miyamoto *et al*., 2011). We found its infection in some chick embryos when etiological studies were conducted on chick embryos of some hatcheries in China in recent years (Liu *et al*., 2006). To analyze the pathogenicity changes of *B. avium*, one isolate

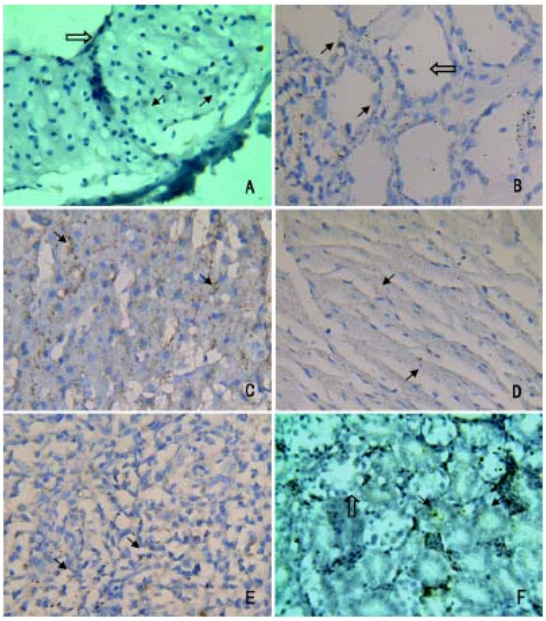

Fig. 3: Colonization of *B. avium* LL09 in different organs (400×). A, Colonization of tracheas by *B. avium* LL09; B, Colonization of lungs by *B. avium* LL09; C, Colonization of livers by *B. avium* LL09; D, Colonization of hearts by *B. avium* LL09; E, Colonization of spleens by *B. avium* LL09; F, Colonization of kidneys by *B. avium* LL09. All of them were detected by indirect immunoenzyme histochemistry. The sections were colored using a DAB staining kit, and then counterstained with hematoxylin. *B. avium* are readily discernible by their distinct brown signals in figures. The colonization sites are marked with solid arrows and specific lesions are marked with empty arrows.

from chick embryo was tested in this experiment. Its colonization pattern in different viscera of SPF chickens was systematically studied under the condition of experimental infection.

We established an indirect immunoenzyme histochemistry method which could localize *B. avium* in chicken tissues. It had the coincidence rate of 100% with results of bacterial isolation. Besides, the exact sites and quantity of *B. avium* in tissues could be observed. Based on the features of specificity, visuality and sensitivity, it was applied for *B. avium* detection in this study.

It was discovered by bacterial isolation and indirect immunoenzyme histochemistry that the infection rates of both tracheas and lungs reached as high as 100% at the initial stage of infection (1-35 d). However, visceral organs of merely 1/3-1/2 of chickens were colonized by *B. avium* LL09 (Table 1), rather than a 100% infection rate. Furthermore, with the enhancement of chicken body immune function and the antibody levels rise (Fig. 2), most bacteria could be cleared away from livers, hearts and spleens (Fig. 1 and Table 1). These results partially explain the reason for high infection and low mortality of Bordetellosis (Jackwood and Saif, 2003).

During infection, *B. avium* was typically found bound to the surface of nasal cavity and the ciliated cells of tracheal epithelium (Temple *et al*., 1998). We also found this appearance in experiment. It mainly adhered to the ciliated tracheal cells until 7 d post-infection, and colonized inner cells of trachea until 21 d post-infection (Fig. 3-A). Lungs had been colonized extensively in less than 7 d post-infection (Fig. 1 and Table 1) and gradually

showed pathological injuries such as congestion, hemorrhage, etc (Fig. 3-B). It indicated that lungs colonization of *B. avium* should happen earlier than the time that the ciliated tracheal cells falling off into lungs after tracheal damages happened. Although *B. avium* would mostly adhere to the ciliated tracheal cells after intranasal infection, a slight amount of bacteria could still directly enter lungs through trachea. They would adhere to alveolar walls, and cause damages to lungs when sufficient in number. It would happen more easily in a severely polluted external environment.

Results of indirect immunoenzyme histochemistry showed that all cases of *B. avium* invasion in viscera were accompanied by severe lung infection (Fig. 3-B). The two patients, who were reported that *B. avium* was isolated from, had cystic fibrosis or chronic obstructive pulmonary disease (Spilker *et al*., 2008; Harrington *et al*., 2009). It indicated that lung was an important carrier to block pathogen invasion. *B. avium*, as an opportunistic pathogen, could not only colonize the upper respiratory tract but could continue to invade visceral organs, especially, when the lungs were severely damaged or suffered from some diseases. Lung infection played a crucial role in continued invasion of *B. avium*.

In conclusion, this study revealed that *B. avium* isolate LL09 had an extremely strong ability of infection and invasion in chickens. It could colonize not only the respiratory tract, but also viscera. The severity of disease caused by *B. avium* correlated completely with its ability to invade into and transfer among different visceral organs after colonizing the respiratory tract.

Acknowledgement: We thank Xiaolin Zhu for revisions in English language. This work was supported by grants from National Natural Science Foundation of China (grant No. 31272595, 30972183 and 30740077) and Youth Innovation Fund of Shandong Agricultural University (grant No. 23821).

REFERENCES

Beach NM, S Thompson, R Mutnick, L Brown, G Kettig, R Puffenbarger, SB Stockwell, D Miyamoto and L Temple, 2012. *Bordetella avium* antibiotic resistance, novel enrichment culture, and antigenic characterization. Vet Microbiol, 160: 189-196.

Guo DQ, C Tang, Q Hai, GQ Shao and H Yue, 2010. Development of a universal plate-agglutination test for detecting *Haemophilus parasuis*. J Vet Sci, 11: 355-357.

Harrington AT, JA Castellanos, TM Ziedalski, JE Clarridge III and BT Cookson, 2009. Isolation of *Bordetella avium* and novel Bordetella strain from patients with respiratory disease. Emerg Infect Dis, 15: 72-74.

Jackwood MW and YM Saif, 2003. Bordetellosis (turkey coryza). In: YM Saif (ed), Diseases of poultry, 11th ed. Iowa State Press, Ames, Iowa, pp: 705-718.

Kirby AE, DJ Metzger, ER Murphy and TD Connell. 2001. Heme utilization in *Bordetella avium* is regulated by Rhul, a heme-responsive extracytoplasmic function sigma factor. Infect Immun, 69: 6951-6961.

Kirby AE, ND King and TD Connell, 2004. RhuR, an extracytoplasmic function sigma factor activator, is essential for heme-dependent expression of the outer membrane heme and hemoprotein receptor of *Bordetella avium*. Infect Immun, 72: 896-907.

Li JX, Y Tang, JY Gao, CH Huang and MJ Ding, 2011. *Riemerella anatipestifer* infection in chickens. Pak Vet J, 31: 65-69.

Liang MF, QY Zhao, GH Liu, SF Yang, XM Zuo, GL Cui, SX Zhong, J Sun, JJ Liu and RL Zhu. 2013. Pathogenicity of *Bordetella avium* under immunosuppression induced by Reticuloendotheliosis virus in specific-pathogen-free chickens. Microbial Pathogenesis, 54: 40-45.

Liu HQ, RL Zhu, QY Sun, XN Hu, JM Bi, HZ Liu, XC Li and W Wang, 2006. Pathogenic studies on the diseases of avian embryo in AA broiler breeder and the detection of its main pathogens by PCR. J Agric Biotechnol, 14: 646-651 (in Chinese).

Loker SB, LM Temple and A Preston, 2011. The *Bordetella avium* BAV1965-1962 fimbrial locus is regulated by temperature and produces fimbriae involved in adherence to turkey tracheal tissue. Infect Immun, 79: 2423-2429.

Miyamoto DM, K Ruff, NM Beach, SB Stockwell, A Dorsey-Oresto, I Masters and LM Temple, 2011. *Bordetella avium* causes induction of apoptosis and nitric oxide synthase in turkey tracheal explant cultures. Microbes Infect, 13: 871-879.

Munir R, M Afzal, M Hussain, SMS Naqvi and A Khanum, 2010. Outer membrane proteins of *B. abortus* vaccinal and field strains and their immune response in buffaloes. Pak Vet J, 30: 110-114.

Murphy ER, RE Sacco, A Dickenson, DJ Metzger, Y Hu, PE Orndorff and TD Connell. 2002. BhuR, a virulence-associated outer membrane protein of *Bordetella avium*, is required for the acquisition of iron from heme and hemoproteins. Infect Immun, 70: 5390-5403.

Nolte FS and AM Caliendo. 2003. Molecular detection and identification of microorganisms. In PR Murray, EJ Baron, JH Jorgensen, MA Pfaller and RH Yolken (ed), Manual of clinical microbiology, 8th ed American Society for Microbiology, Washington, DC, pp: 234-256.

Odugbo MO, U Musa, SO Ekundayo, PA Okewole and J Esilonu, 2006. *Bordetella avium* infection in chickens and quail in Nigeria: preliminary investigations. Vet Res Commun, 30: 1-5.

Probst HC, K Tschannen, B Odermatt, R Schwendener, RM Zinkernagel, M Van Den Broek, 2005. Histological analysis of CD11c-DTR/GFP mice after in vivo depletion of dendritic cells. Clin Exp Immunol, 141: 398-404.

Raffel TR, KB Register, SA Marks and L Temple, 2002. Prevalence of Bordetella avium infection in selected wild and domesticated birds in the eastern USA. J Wildlife Dis, 38: 40-46.

Register KB and AG Yersin. 2005. Analytical Verification of a PCR Assay for Identification of *Bordetella avium*. J Clin Microbiol, 43: 5567-5573.

Sacco RE, KE Nestor and RA Kunkle. 2000. Genetic variation in response of turkeys to experimental infection with *Bordetella avium.* Avian Dis, 44: 197-200.

Sebaihia M, A Preston, DJ Maskell, H Kuzmiak, TD Connell, ND King, PE Orndorff, DM Miyamoto, NR Thomson, D Harris, A Goble, A Lord, L Murphy, MA Quail, S Rutter, R Squares, S Squares, J Woodward, J Parkhill and LM Temple. 2006. Comparison of the genome sequence of the poultry pathogen *Bordetella avium* with those of *B. bronchiseptica, B. pertussis, and B. parapertussis* reveals extensive diversity in surface structures associated with host interaction. J Bacteriol, 188: 6002-6015.

Shelton CB, LM Temple and PE Orndorff, 2002. Use of bacterio-phage Ba1 to identify properties associated with Bordetella avium virulence. Infect Immun, 70: 1219-1224.

Spears PA, LM Temple, DM Miyamoto, DJ Maskell and PE Orndorff, 2003. Unexpected similarities between *Bordetella avium* and other pathogenic bordetellae. Infect Immun, 71: 2591-2597.

Spilker T, AA Liwienski and JJ Lipuma, 2008. Identification of Bordetella spp. in respiratory specimens from individuals with cystic fibrosis. Clin Microbiol Infec, 14: 504-506.

Suresh P, 1993. Detecting *Bordetella avium* in tracheal sections of turkeys by monoclonal antibody-based indirect fluorescence microscopy. Avian Pathol, 22: 791-795.

Temple LM, AA Weiss, KE Walker, HJ Barnes, VL Christensen, DM Miyamoto, CB Shelton and PE Orndorff, 1998. *Bordetella avium* virulence measured in vivo and in vitro. Infect Immun, 66: 5244-5251.

Temple LM, DM Miyamoto, M Mehta, CM Capitini, VS Stetina, HJ Barnes, VL Christensen, JH Horton, PA Spears and PE Orndorff, 2010. Identification and characterization of two *Bordetella avium* gene products required for hemagglutination. Infect Immun, 78: 2370-2376.

Ünver B and SÜ Saraydın, 2012. Macroscopical and histological analysis of gonadal development of *Squalius cephalus* (l., 1758) in Tödürge Lake, Turkey. Pak Vet J, 32: 55-59.

Vichi J, M Colás, I Espinosa and S Martínez, 2011. Isolation of *Bordetella avium* in White Leghorn hens in Cuba: first report. Rev Salud Anim, 33: 136.

Yang PP, RD Ma, X Zhao and RL Zhu, 2012. Comparative serological and random amplified polymorphic DNA typing for *Bordetella avium* isolates in China. Pak Vet J, 32: 552-556.

Zhu RL, SX Zhang and KX Tang, 1991. Primary study on the disease caused by *Bordetella avium* in chickens. J Shandong Agric Univ, 22: 92-94 (in Chinese).