

## **An Outbreak of *Avibacterium paragallinarum* serovar B in a Thai Layer Farm**

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### ***Abstract***

*Avibacterium paragallinarum* serovar B was diagnosed and revealed in a layer farm. The infected chickens showed mucous nasal discharge, facial edema and conjunctivitis. Egg production also decreased dramatically. Necropsy findings showed lesions of conjunctivitis and edematous tissue around the eyes. The infraorbital sinuses were filled with a caseopurulent mass and tracheitis. Bacterial isolation and identification was performed and confirmed by PCR technic. A hemagglutination inhibition test using specific antibodies against serovar B revealed the *Avibacterium paragallinarum* serovar B infection. A pathogenicity test done in 10 layer chickens, showed clinical signs of nasal discharge, facial edema and conjunctivitis. This is the first report of an outbreak of infectious coryza caused by *A. paragallinarum* serovars B in Thailand.

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**Keywords:** *Avibacterium paragallinarum*, layer farm, serovar B

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## บทคัดย่อ

### การระบาดของ *Avibacterium paragallinarum* ซีโรวาร์บี ในฟาร์มไก่ไข่ในประเทศไทย

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ไก่ไข่ที่ติดเชื้อมีน้ำมูกเหนียวข้น หน้าบวม เยื่อตาขาวอักเสบ และไข่ลดลงอย่างมาก ผลการชันสูตรพบ เยื่อตาขาวอักเสบ เนื้อเยื่อรอบดวงตาบวม น้ำ มีก้อนหนองในไซนัสใต้ตาและหลอดลมอักเสบ ทำการเพาะแยกเชื้อและพิสูจน์เชื้อแบคทีเรีย และยืนยันโดยวิธีการพีซีอาร์ ทำการแยกซีโรวาร์โดยวิธีการยับยั้งการตกตะกอนกับเม็ดเลือดแดง โดยใช้แอนติบอดีเฉพาะต่อเชื้อ *A. paragallinarum* ซีโรวาร์บี พบการติดเชื้อ *A. paragallinarum* ซีโรวาร์บี ทำการทดสอบการเกิดพยาธิสภาพ ในไก่ไข่ 10 ตัว พบว่าไก่ไข่แสดงอาการมีน้ำมูก หน้าบวม และเยื่อตาขาวอักเสบ รายงานนี้เป็นรายงานการระบาดของโรคหวัดหน้าบวมที่เกิดจากเชื้อซีโรวาร์บีครั้งแรกในประเทศไทย

**คำสำคัญ:** เอดิแบคทีเรีย พาราไกลลินารุม ฟาร์มไก่ไข่ ซีโรวาร์ บี

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## Introduction

Infectious coryza is an acute respiratory disease in chickens. The disease has worldwide economic recognition and causes infection in both broiler and layer flocks. The disease is caused by gram negative bacteria *Avibacterium paragallinarum* which are classified into three serovars (A, B and C) using a slide agglutination test (Page, 1962). There is no cross protection between serovars, so commercial vaccines combine many serovars and including bivalent (serovar A and C) vaccine, trivalent (serovar A, B and C) vaccine or tetravalent (serovar A, B, C and B variant) vaccine. Sariano et al. (2004) found that cross protection within serogroup A was more than serogroup C. Different serovars between the vaccine and field strains may be the cause of failure in vaccination because of the lack of no cross protection properties. Thus, the suitable selection of a vaccine application in each infected area is the key to success in disease prevention and control.

Some literature indicated that the pathogenic serovars of the bacteria are A and C whereas serovar B is not clearly understood regarding its pathogenicity (Page, 1962; Kume et al., 1980; Thornton and Blackall, 1984). Yamaguchi et al. (1990) reported that serovar B is pathogenic in Argentina. The outbreaks of infectious coryza in some countries such as South Africa and the United States of America have occurred under the suspicion of serovar B being involved (Jacobs et al., 2003). The epidemiology of serovar prevalence of *A. paragallinarum* in each infected area is a crucial criterion of the type of vaccine application for providing suitable protection. In Thailand, serovar A and C outbreaks have been reported (Neramitmansuk et al., 1995; Chukiatsiri and

Chansiripornchai, 2008; Chukiatsiri et al., 2009). *A. paragallinarum* infection has been found in both Thai industrial and native chickens although the chickens have received vaccine according to appropriate programs (Neramitmansuk and Neramitmansuk, 1985; Chukiatsiri et al., 2008). Herein, the first report of an outbreak of the clinical and pathological findings of *A. paragallinarum* serovar B infection in Thailand is revealed.

## Case History

In July 2009, 9 birds from a layer farm in the province of Chiang Mai were sent to the Animal Diagnostic Centre, Faculty of Veterinary Medicine, Chiang Mai University, Thailand. These birds exhibited severe respiratory problems such as respiratory distress, nasal discharge and facial edema. Also a decrease in egg production was found. History taking revealed that the farm consisted of 3 houses of pullets (approximately 8,000 birds each) and 2 houses of layers (approximately 32,000 birds each). The disease had occurred 2 days prior to submission and previous medication had not been given. Egg production decreased from 90.2% to 83.0% in house 1 and 89.7% to 85.1% in house 2. Morbidity and the mortality were 3% and less than 1%, respectively. These chickens had been previously vaccinated against infectious coryza using a bivalent vaccine (serovar A and C). On examination, the layers were depressed and had mucous discharge, conjunctivitis and facial edema.

## Results and Discussion

**Necropsy:** Five birds were euthanized by cervical dislocation. Conjunctivitis and subcutaneous edema around the eyes were found. Infraorbital sinuses were deposited with caseous masses. Tracheitis and mucous secretion were found.

**Bacterial isolation and identification:** Bacteria were swabbed from the infraorbital sinuses and cultured on a blood agar plate that had been crossed streaked with *Staphylococcus aureus*. After being incubated in 5%CO<sub>2</sub> at 37°C for 24 hrs, the bacterial colonies showed satellite growth. The *A. paragallinarum* isolates were confirmed by HPG-2 PCR primer (Chen et al., 1996). The field strain indicated serovar B by a hemagglutination inhibition (HI) test using a specific antiserum of serovar B. A cross reaction of this isolate was not found with the specific antisera against serovar A and C (Animal Research Institute, Queensland, Australia).

**Serology:** Thirty birds per house were randomly sampled and the blood samplings were done twice. The first time was performed at the onset of the disease (July 2009) and then the second was performed 4 weeks later (August 2009). Reference antigen B was used to detect antibody titer to serovar B by HI test following the Kitasato institute method. Briefly, the tested sera were absorbed by adding 10% (v/v) glutaraldehyde (GA)-fixed chicken erythrocytes to make a final dilution of 1:5. The mixture was allowed to stand for 2 hrs at room temperature or overnight at 4°C, then it was centrifuged and the supernatant was used as the fivefold-diluted serum for the HI test. The fivefold diluted serum was diluted by a twofold dilution method using 0.1% bovine serum albumin in PBS to give dilutions of 1/10-1/640 (each well contained 0.2 ml of each diluted serum). The same amount (0.2 ml) of the antigen with 4 hemagglutinating units/0.2 ml were added to each well. The mixture was shaken well and allowed to stand for 20 min at room temperature. Finally, 0.4 ml of 1% (v/v) GA-fixed chicken erythrocyte was added and shaken well. The mixture was allowed to stand for 60 min at room temperature before reading. The maximum serum dilution completely inhibiting hemagglutination was regarded as an HI titer. The antibody titer of each sample of the first blood sampling against serovar B was less than the lowest level of antibody detection. For the second blood sample collection, most of the samples showed detectable antibody titers against serovar B and one sample revealed a four time higher antibody titer against serovar B than the lowest antibody detection level.

**Challenge test:** The isolated colonies of *A. paragallinarum* serovar B were inoculated in chicken meat infusion (CMI) broth and incubated at 37°C for 24 hrs and inoculated into 7 days old embryonated eggs to increase their pathogenicity. The embryonated eggs died within 24 hrs and the egg yolks were collected and inoculated in CMI broth for bacterial

growth. Then, 4 week old female layer chickens were used for a challenge study by intranasal inoculation of both sides with 100 µl of bacterial concentration at 10<sup>8</sup> cfu/ml. The chickens showed clinical signs such as a swollen face, nasal discharge and conjunctivitis 4 days after being challenged and were euthanized 7 days after being challenged. Eight of the ten chickens were isolated *A. paragallinarum* serovar B from the infraorbital sinus.

Although some reports have shown serovar B not to be clearly pathogenic in chicken (Page, 1962; Kume et al., 1980; Thornton and Blackall, 1984), many recent reports have reviewed the pathogenicity of serovar B in many countries including the United States of America, Ecuador, Argentina and Zimbabwe (Yamaguchi et al., 1990; Poernomo et al., 2000; Zhang et al., 2003). In Thailand, there have been a few reports regarding infectious coryza and a previous report showed pathogenic *A. paragallinarum* to only serovar A and C (Neramitmansuk et al., 1995). This article is the first report to have revealed the detection of *A. paragallinarum* serovar B in Thailand. Furthermore, this isolate can cause pathogenicity in infected chickens. The result may help poultry veterinarian practitioners and farm owners to select suitable vaccines to prevent outbreaks in their supervised farms. In this case, the chickens were vaccinated with infectious coryza using bivalent vaccine (serovar A and C), however the birds were infected with *A. paragallinarum* serovar B. Therefore, a suitable vaccine should be selected to promote protection against *A. paragallinarum* infection.

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