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An outbreak of chronic fowl cholera in broiler breeder chickens in Korea

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Abstract

Fowl cholera is a contagious acute and chronic disease caused by *Pasteurella multocida* in both domesticated and wild birds. Acute fowl cholera in both chickens and wild birds has recently been documented in Korea, but the chronic form has not been reported in Korea until now. This study describes the first outbreak of chronic fowl cholera in 13-week-old Arbor Acre broiler breeder chickens submitted to the College of Veterinary Medicine, Kyungpook National University in April 2006. The clinical signs of the affected flock of 9,621 chickens were lameness caused by swollen hock joints, diarrhea, ruffled feathers, and an average weekly mortality of 1.0%. At necropsy, purulent or caseous exudates were found in the hock and wing joints, humerus, and eyes, and severe pneumonia and pericarditis were discovered. Eleven bacterial isolates obtained from the liver, joint, infraorbital sinus and sternal bursa of the submitted chickens were all identified as *Pasteurella multocida* based on their physiological and biochemical characteristics. Five isolates were examined for antimicrobial susceptibility against 21 different antimicrobial agents including ampicillin. All were resistant to kanamycin, neomycin, and streptomycin, and some were resistant to gentamicin. The tested isolates were all susceptible to the other 17 antimicrobial agents. All 11 isolates were capsular serogroup A based on multiplex polymerase chain reaction. In addition, two of five isolates used in the antimicrobial susceptibility test were identified as somatic serotype 1 by an agar gel diffusion precipitin test, while the others were non-typable.

Key words : Chronic fowl cholera, *Pasteurella multocida,* Capsular serogroup, Somatic serotype

INTRODUCTION

Fowl cholera (FC) is a contagious disease in both domesticated and wild birds caused by the bacterium *Pasteurella*(*P.*) *multocida*. In domesticated birds, FC causes significant economic losses worldwide (Christensen et al, 2008; Gilsson et al, 2003). Turkeys and waterfowl are most affected, and death from FC in chickens usually occurs in laying flocks, because birds of this age are more susceptible than younger chickens (Gilsson et al, 2003).

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Acutely affected birds typically die from septicemia and exhibit hemorrhages in the heart and lungs. The liver usually contains multiple small necrotic foci. Chronic FC is characterized by localized infections in the wattle, infraorbital sinus, sternal bursa, hock and wing joints, and foot pads. Chronic FC may follow an acute stage of the disease or result from infection with organisms of low virulence (Christensen et al, 2008; Gilsson et al, 2003; Timoney et al, 1992).

P. multocida is a gram-negative rod with bipolar staining that affects cattle (Frank, 1989), pigs (Blackall et al, 2000), rabbits (Langan et al, 2000), and humans (Weber et al, 1984), in addition to fowl (Christensen et

al, 2008). *P. multocida* includes capsulated and non-capsulated types, with the former being more virulent than the latter (Boyce and Adler, 2000; Chung et al, 2001). *P. multocida* can be differentiated serologically by capsular antigens into serogroups A, B, D, E, and F (Rimler and Rhoades, 1987), and also by somatic antigens into somatic serotypes 1～16 (Borgden et al, 1978). Among the five capsular serogroups, B and E cause hemorrhagic septicemia in cattle and wild ruminants (De Alwis, 1992), and serogroup D causes atrophic rhinitis in pigs (Quinn et al, 1994). In poultry, capsular serogroup A causes fowl cholera (Timoney et al, 1992). All somatic serotypes have been isolated from birds. Among sixteen somatic serotypes, 1, 3 and 4 are most frequently reported (Adler et al, 1999).

FC occurs sporadically or enzootically in most countries (Gilsson et al, 2003). FC has recently been documented in both domesticated and wild birds in Korea. A significant number of wild birds died in the Cheon-su bay located in Chungcheongnam province, in October, 2000 (Kwon and Kang, 2003). In domesticated birds, acute FC was reported in broiler breeder farms in October, 2000 and February, 2001 (Woo and Kim, 2006), but the chronic type has not been reported in Korea until now.

In this study, we confirm the chronic FC outbreak characterized by a localized infection of low mortality in broiler breeder chickens in Korea.

MATERIALS AND METHODS

Chickens

Thirteen-week-old Arbor Acre broiler breeder chickens, suffering from lameness along with the weekly mortality rate of around 1%, were submitted to the College of Veterinary Medicine, Kyungpook National University from a broiler breeder farm located in Gyeonggi province on April 2006.

Necropsy and histopathology

The dead and live chickens submitted for diagnosis of diseases were necropsied to find specific gross lesions. After a postmortem examination of them, the liver, lungs, heart, eyes and joints were removed and then fixed in 10% neutral buffered formalin. All tissue samples were embedded in paraffin and sectioned at 5 μm, and stained with hematoxylin and eosin (H&E).

Bacterial isolation and examination

Specimens of the hock joints, liver, infraorbital sinus and sternal bursa were aseptically collected after necropsy. They were streaked on blood agar plate (BA, Difco, USA) and MacConkey agar plate (MAC, Difco, USA), and these plates were incubated at 37°C during approximately 48 hr. Colonies suspected to be *P. multocida* from BA were stained by Gram and Giemsa, and then according to the method of Barrow and Feltham (1993), the biochemical and physiological tests including oxidase, catalase, indole, urease, carbohydrate fermentation and motility were conducted.

Antimicrobial susceptibility test

Among 11 *P. multocida* isolated in this study, five were randomly selected by their different biochemical properties. They were tested for antimicrobial susceptibility using the disc diffusion method in accordance with National Committee for Clinical Laboratory Standards (NCCLS, 2003). The isolates were tested against 21 antimicrobials: amoxicillin/clavulanic acid, ampicillin, ciprofloxacin, cefoperazone, cefoxitin, cephalothin, colistin, doxycycline, gentamicin, kanamycin, neomycin, norfloxacin, ofloxacin, polymixin B, streptomycin, trimethoprim/sulfamethoxazole, tetracycline (Becton Dickinson, USA), enrofloxacin (Bayer, Germany), lincomycin/spectinomycin, cetiofur, and tiamulin (Rosco, Denmark). The results were interpreted according to the guidelines of the particular manufacturer.

Capsular polymerase chain reaction (PCR) typing and somatic serotyping

Capsular typing was performed by multiplex capsular PCR with the capsule-specific primers pairs (CAPA,

CAPB, CAPD, CAPE, and CAPF) designed by Townsend et al (2001). Colonies were transferred to an eppendorf tube filled with distilled water and boiled to prepare DNA templates for PCR. The amplification conditions were 95°C for 5 min as the primary denaturation step, 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension step of 72°C for 5 min. Amplification products were provisionally identified by their size in ethidium bromide-stained agarose gels. The positive amplified PCR product was analyzed with an automated DNA sequencing system.

The somatic serotyping of the 5 isolates used in antimicrobial susceptibility test was performed using the agar gel diffusion precipitin test in accordance with the method of Heddleston et al. (1972) using antisera from the National Veterinary Service Laboratories (NVSL, USA). Also, somatic serotyping test was entrusted to the NVSL, to secure accuracy.

RESULTS

History and clinical signs

The affected flock consisted of 8,260 pullets and 1,361 roosters. The average weekly mortality of this flock was approximately 1.0% over than 7 week period from 9 to 15 weeks of age, with peak mortality of 1.5% for 1 week as shown in Table 1. Culled chickens exceeded the number of dead chickens. The clinical signs were diarrhea, ruffled feathers, ocular opacity, and lameness caused by swollen hock joints. The clinical signs temporarily improved after gentamicin injection and oxytetracycline administration.

Gross findings and microscopic lesions

In the postmortem, the unilateral or bilateral swollen orbit and caseous exudates of eyeball (Fig. 1A) were revealed. Mucus tracheal exudates were also observed. The purulent or caseous exudates were seen in hock and wing joints (Fig. 1B), and humerus. Also petechial or ecchymotic hemorrhages were found in the epicardium. The liver was swollen with multiple ecchymotic hemorrhages and small necrotic foci. The hemorrhage and hyperemia were observed in the lungs, and severe fibrous exudates were seen on the pleural surface of the lungs.

The most prominent microscopic lesions of the dead chickens were observed in the hock joints, eye, lungs, liver and heart. Degenerative heterophils, fibrin and eosinophilic materials were observed in the articular cavity of hock joints (Fig. 2) and the anterior chamber of the eye, and the granulomas were scattered through the lung parenchyma. In the liver, there were the atrophy of hepatic lobules because of increased connective tissue, multiple necrotic foci with bacteria and heterophilic infiltration. Heterophils, lymphocytes and macrophages had infiltrated the myocardium, and granulomatous inflammation composed of cell debris and multinucleated giant cells were observed between the pericardium and epicardium.

Table 1. The weekly mortality rate of broiler breeder chickens affected by chronic fowl cholera

Age (weeks)	Pullet*			Rooster*		
	Flock size	No. of dead and culling chickens	Mortality rate (culling and death)	Flock size	No. of dead and culling chickens	Mortality rate (culling and death)
9	8,260	91	1.1%	1,361	13	1.0%
10	8,169	64	0.8%	1,348	6	0.4%
11	8,105	82	1.0%	1,342	16	1.2%
12	8,023	74	0.9%	1,326	16	1.2%
13	7,949	120	1.5%	1,310	13	1.0%
14	7,829	94	1.2%	1,297	6	0.5%
15	7,735	61	0.8%	1,291	19	1.5%

*Breeder: Arbor Acre broiler breeder chicken.

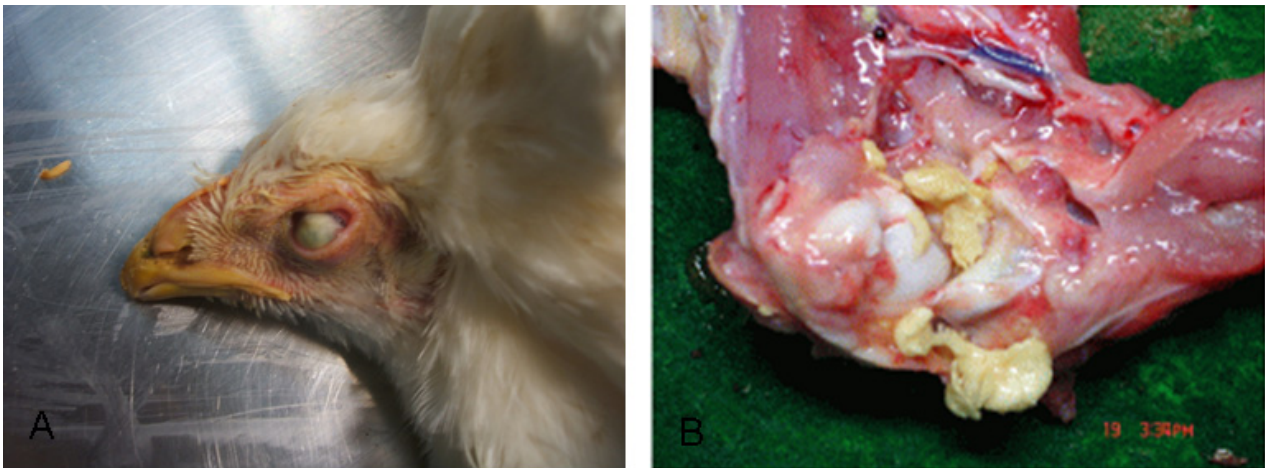

Fig. 1. Gross lesions of broiler breeder chickens caused by fowl cholera. (A) Ocular caseous exudates. (B) Yellowish-colored caseous exudates of a wing joint.

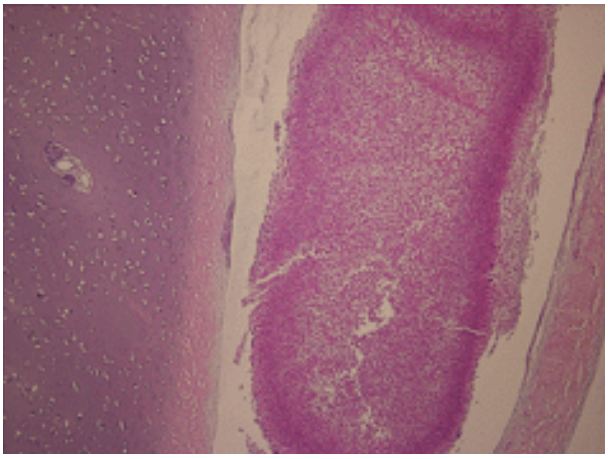

Fig. 2. Microscopic lesions of the hock joint from chicken characterized by infiltration of degenerative heterophils, fibrin, and eosinophilic materials in the articular cavity; H&E stain, ×100.

Isolation and identification of *P. multocida*

Eleven isolates were obtained from hock joints (7 isolates), liver (2 isolates), sternal bursa (1 isolate) and infraorbital sinus (1 isolate). They grew well on BA without hemolysis, but not on MAC. They showed bipolar staining, were gram-negative rods, and were not motile. They produced oxidase, catalase, indole and ornithine decarboxylase, but did not produce urease and hydrogen sulfide. In the carbohydrate fermentation test, all isolates fermented dextrose, fructose, galactose, mannose, sucrose, trehalose, and xylose, but not dulcitol, inositol, inulin, lactose, maltose, and rhamnose. Mannitol and sorbitol fermentation was observed in most isolates, but the fermentation of arabinose and salicin was rarely observed. Based on the morphologic, biochemical, and physiological characteristics, all isolates were identified as *P. multocida.*

Antimicrobial susceptibility

As the results of antibiogram of 5 isolates against 21 different antimicrobial agents, they were resistant to kanamycin, neomycin and streptomycin, and susceptible to amoxicillin/clavulanic acid, ampicillin, ciprofloxacin, colistin, norfloxacin, ofloxacin, trimethoprim/sulfamethoxazole, tetracycline, cephalothin, polymixin B, cefoperazone, doxycycline, enrofloxacin, cefoxitin, lincomycin/spectinomycin, cetiofur and tiamulin. Three isolates of them were not susceptible to gentamicin.

Capsular serogroup and somatic serotype

As the result of capsular PCR typing, all 11 isolates simultaneously produced 1044 bp and 460 bp PCR product known as the capsular serogroup A. As the result of somatic serotyping by the agar gel diffusion precipitin test, of 5 isolates used in antimicrobial susceptibility test, two were somatic serotype 1, but the others were non-typable. Also, they were given to the NVSL to obtain the accurate results. The somatic serotype confirmed by the NVSL was consistent with our results.

DISCUSSION

P. multocida is a facultative anaerobic, gram-negative, and non-motile bacterium that causes FC in wild and domesticated birds. It was first noted in the blood of birds in 1877 (Weber et al, 1984). The disease usually occurs in birds in two forms: an acute septicemia with high morbidity and mortality rates, and a chronic localized infection of joints and sinuses (Gilsson et al, 2003). Chronically infected birds are considered to be a major source of transmission and the most likely routes of FC transmission among birds are through direct contact or via contaminated water (Christensen and Bisgaard, 2000; Curtis and Ollerhead, 1981).

The chickens submitted by the affected broiler breeder farm in this study exhibited lesions of edema, and purulent and caseous exudates in the hock and wing joints. These lesions may appear for colibacillosis (Barnes et al, 2003), salmonella infection (Gast and Shivaprasad, 2003) and staphylococcosis (Andreasen, 2003). A differential diagnosis was made through various biochemical and physiological tests of bacteria isolated from the lesions and the organism was definitively identified as *P. multocida*.

In Korea, FC recently occurred in both poultry and waterfowl. Previously, FC occurring in Baikal teals was characterized by high mortality rates and hemorrhage of the heart, liver, and small intestine (Kwon and Kang, 2003). Also, in case of chickens from broiler breeder farms in Gyeonggi province and Chungcheongnam province, perihepatitis and severe pneumonia were found to be characteristic lesions (Woo and Kim, 2006). In this study, however, FC was characterized by severe lameness due to arthritis and inflammation of conjunctiva, along with a low mortality rate. The present findings differ from the clinical signs and gross findings of FC observed previously in waterfowl and chickens in Korea. On the basis of the characteristic clinical signs and pathologic findings, the disease that occurred in the chickens was confirmed as the first outbreak of chronic FC in Korea.

Presently, all 11 isolates fermented carbohydrates including dextrose, fructose, galactose, mannose, sucrose, trehalose, and xylose, while more varied fermentation of arabinose, mannitol, salicin, and sorbitol was observed. These results are similar with isolates from chickens (Woo and Kim, 2006), but differ from isolates from waterfowl, which did not ferment trehalose (Kwon, 2003). Distinct differences between carbohydrate fermentation of the isolates in waterfowl and poultry are evident.

Among the 11 *P. multocida* isolated in this study, five isolates were randomly selected based on different biochemical properties. All isolates displayed resistance to kanamycin, neomycin, and streptomycin. Some isolates were resistant to gentamicin, yet susceptible to all other antimicrobials. Previously-tested isolates from chickens in Korea displayed resistance to kanamycin, neomycin, tobramycin, gentamicin, oxytetracycline, tetracycline, and doxycycline (Woo and Kim, 2006), and isolates from poultry in Indonesia displayed resistance to lincomycin and sulfadiazine (Jones et al, 2001). However, isolates from waterfowl displayed low resistance to gentamicin and kanamycin, and a high susceptibility to other antimicrobial drugs (Kwon, 2003). The wider spectrum of antimicrobial resistance of poultry isolates, as compared to isolates from waterfowl may reflect increased exposure to antimicrobial drugs in poultry.

The capsular serogroups identified in birds so far are A, B, D, and F (Boyce and Adler, 2000), and FC is known to involve mainly the capsular serogroup A (Timoney et al, 1992). Somatic serotypes 1, 3, and 4 are the most prevalent serotypes in birds (Adler et al, 1999). Also, many *P. multocida* strains that have multiple somatic antigens are commonly found in avian hosts (Windingstad et al, 1988). In this study, the capsular serogroup of the isolates was A, and two of the five randomly selected isolates were somatic serotype 1. These observations are consistent with previous descriptions that the A: 1 serotype is the most common serotype in birds (Adler et al, 1999; Timoney et al, 1992). This serotype was recently reported in the isolates from turkeys in the United States (Aye et al, 2001) and from layer chicken in Pakistan (Arshad et al, 2003). On the other hand, the serotype of the isolates in waterfowl was A: 1×12×14 (Kwon and Kang, 2003), and in broiler breeder chickens was reported to be A: 10×11 (Woo and Kim, 2006) in Korea. Consequently, there was no corre-

lation between the antigens of isolates from our study and previous work in Korea. The nature of the influx route of *P. multocida* serotype A: 1 into Korea requires further research.

The outbreak of chronic FC in Korea could be a matter of significant concern to the poultry in the near future. We should effectively control chronic FC to minimize the damage and economic loss incurred by farms. Also, it will be needed to make a wider and deeper study on epidemiology and preventive method such as vaccine development.

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