大韓獸醫學會誌(2005) 第45卷 第1號
Korean J Vet Res(2005) 45(1) : 71~74

Occurrence of *Pseudomonas aerusinosa* infection in the broilers in Korea

Seong-joon Joh, Min-chul Kim, Yong-kuk Kwon*, Jae-hong Kim

Avian Disease Division, National Veterinary Research and Quarantine Service, MAF, Anyang 430-824, Korea
(Accepted: October 18, 2004)

Abstract : *Pseudomonas aerusinosa* infection was diagnosed in broiler chicks, and was submitted to the National Veterinary Research and Quarantine Service in Korea. The total mortality rate was about 1,500 birds out of 22,000 broilers. Clinically, affected birds showed clinical signs including depression and anorexia with lameness and trembling of the leg. At necropsy, the dead broilers appeared to have omphalitis, yolk sac infection, fibrinous epicarditis, and fibrinous exudates in liver with swollen hock joint. Microscopically, there were multiple necrotic foci in the liver, fibrinous exudates in the heart, and infiltration of heterophils into the joint spaces of the hock joint. *Pseudomonas aerusinosa* was isolated from the heart, liver and hock joint, and the isolate was named P-200. In effort to estimate the virulence of P-200, 1-day-old chicks were challenged intramuscularly and intrayolksacally with the isolate. On the basis of mortality rate, the isolate P-200 was found to be highly virulent. This is the first report of an occurrence of *Pseudomonas aerusinosa* infection in broilers in Korea.

Key words : broiler, chick, Korea, *Pseudomonas aerusinosa*, virulence test

Introduction

Pseudomonas *spp* can infect the young and growing poultry locally or systemically by attacking or invading fertile eggs, which cause death of embryos and newly hatched birds [2, 3, 6]. The organism is an opportunistic and ubiquitous, which often exists in decaying vegetation, soil, water, and other humid environments [1, 2, 3, 5].

P. aerusinosa is a motile, gram-negative, non-spore-forming rod which occurs usually in single or in short chains. The organism is a strict aerobe that grows readily on common bacteriologic media, usually producing a water-soluble green pigment composed of fluorescein and pyocyanin [2]. Although birds of any age can be infected with the organism, young birds usually are the most susceptible. The clinical signs include lameness; incoordination; swelling of head, wattles, and hock joint or footpads; diarrhea, and septicemia [2]. Lesions are consistent with clinical findings and include subcutaneous edema; exudates in affected joints; inflammation of serous membranes; swelling and necrotic foci in liver, spleen, kidney and brain [2].

The present study describes early infection of *P. aerusinosa* in young broilers, and evaluated virulence of the bacteria for chicks.

Materials and Methods

Chickens

For virulence test of the isolate in broilers, one hundred 1-day-old broilers (Ross) were obtained from healthy commercial flocks with no previous history of infectious disease. They were fed feed and water provided with *ad libitum*. They were divided two inoculation and two control groups.

Bacterium

The isolate was grown on 5% sheep BA plates at 37°C for 24 hr in aerobic conditions. These cultures were inoculated on tryptic soy broth and diluted with phosphate-buffered saline, pH 7.4, to the desired range by a standard curve developed with optical density and bacterial counts of several different dilutions. The exact

*Corresponding author: Yong-kuk Kwon
Avian Disease Division, National Veterinary Research and Quarantine Service, MAF, Anyang 430-824, Korea
[Tel: +82-31-467-1805, Fax: +82-31-467-1803, E-mail: kwonyk@nvrqs.go.kr]

Table 1. Comparisons on virulence of P-200 isolate, *Pseudomonas aerusinosa*, for 1-day-old chicks with two different inoculation routes

Inoculum	Inoculation Route*	Dose of challenge organism (CFU/bird)	Mortality (Number of chicks died)			
			Within 2 DPI**	Between 2 and 3 DPI	Between 3 and 14 DPI	Total number of dead/ Tested (%)
P-200	IM	1×10^3	6	4	6	16/40 (40)
	IY	1×10^3	37	1	–	38/40 (95)
PBS	IM	–	–	–	–	0/10 (0)
	IY	–	–	–	–	0/10 (0)

*IM: intramuscular, IY: intrayolksac
**DPI: Days post inoculum

concentration of bacteria was determined by plate count procedures.

Virulence test

The bacterial concentration was determined by serial dilution in phosphate-buffered saline (PBS) and inoculation on plate count agar (Difco). The diluents with titer of 1.0×10^3 CFU/m*l* were used for broilers challenge (Table 1). Two test groups and one-control group, which consisted of 40 broilers of each, were used for the virulence test. All broilers were challenged through intramuscular (IM) or yolk sac route (IY) with 0.1 m*l* of the diluents, followed as previously described [7]. The control birds were inoculated with sterile PBS.

Results

Case history

On the June 16th, 2003, a disease characterized by depression, ruffled feathers, lameness and dehydration occurred in a broiler farm, which consist of 22,000 birds and located in middle part of Korea, Chungbuk provinces. Ten 8-day-old broilers were submitted to the Avian Disease Division, National Veterinary Research and Quarantine service, and were diagnosed as *P. aerusinosa* infection.

The birds have been hatched by an integrated broiler company and were directly placed at the farm, one day old. Unusual mortality was first found in stock, 3 days later. During five-day period in 3 to 7 day old stocks, the total mortality was 1,500 broilers.

Gross and histopathology

All the birds submitted generally showed to be dehydrated, and were in poorly physical conditions. The postmortem findings included inflammation of navel, distended and hyperemic yolk sac, and yellowish fibrinous exudates on the pericardium, the serosal surface of liver and intestine, and within peritoneum. The spleen was severely enlarged with multiple mottled foci on the surface. There was severely swollen hock-joint with purulent exudates, as shown in Fig. 1. In the abdominal cavity, mal-absorbed and decayed yolks were also present.

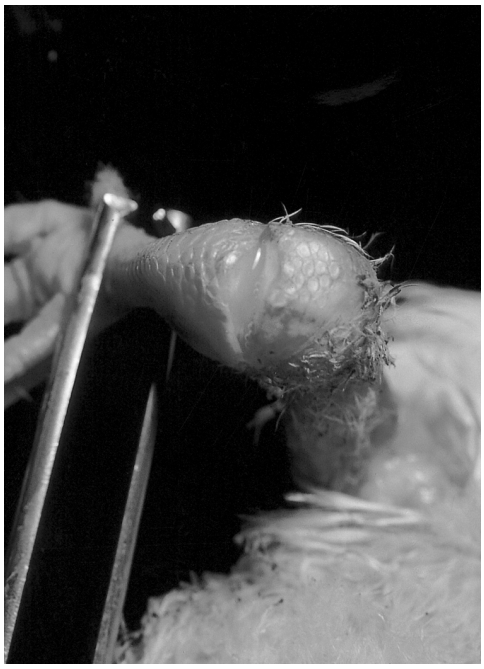

Fig. 1. Purulent exudates in the hock joint of broiler infected with P-200 isolate.

The prominent microscopic lesions of the dead broilers were found in the liver, heart, joint and spleen, which were findings that were compatible with the gross changes. In the liver, there were multiple foci of coagulative necrosis with intra lesion bacteria and heterophilic infiltration. In addition, the serosal surface was covered with fibrino-purulent exudates. Diffuse fibrinous exudates with bacterial colonization were also found in the epicardium. Decreased lymphocytes and moderately increased reticulocytes were the major changes in the spleen. In the hock-joint, there were inflammatory responses characterized by infiltration of heterophils and fibrin into joint spaces and along with tendon sheaths.

Microbiology and antimicrobials sensitivity test

The bacteria, morphologically gram negative, rod, and occurring beta-hemolysis on blood agar (BA), were isolated from the liver, heart and hock joint of broilers. One colony from each bacteria, which was isolated from individual organs was selected as a representative isolate and was tested for biochemical properties, which coincided well with those of *P. aerusinosa* [2]. We identified the isolate with API 20NE test strips (bioMerieux, Marcy l'Etoile, France).

Antibiotic sensitivity tests performed on Mueller Hinton agars (MHA) revealed that P-200 isolate was sensitive to amikacin, colistin, gentamycin, polymyxin B and tobramycin, but resistant to amoxicilin-clavulanic acid, ampicillin, ciprofloxacin, trimethoprime/sulfamethoxazole, kanamycin, neomycin, norfloxacin, ofloxacin, and oxytetracycline.

Virulence test for commercial broilers

The degree of virulence varied greatly influenced with challenge routes. Table 1 shows the number of deaths resulting from the IM or IY exposure with 1.0×10^3 CFU/m*l* concentrations of the P-200 isolate. The mortality rate was 40% and 95% in the birds inoculated with IM and with IY, respectively. Postmortem findings in the dead broilers were congestion and enlargement of liver, peritoneal fluid in the abdomen, epicarditis, omphalitis, and some solidified yolks or both of them. But swelling of hock joint, which was found in broilers in field cases were not observed. No lesions were observed in the birds infected, but not dead or uninfected as control group.

A microbiological examination was performed from the liver and yolk sac of the broilers that died after IM or IY exposure. The results indicated that all of them were *P. aerusinosa*.

Discussion

This case was diagnosed as systemic disease caused by *P. aerusinosa* in the young broiler chicks. In addition, the signs of the disease in the birds that were observed on a gross and histopathological level were very similar to those of caused by other Pseudomonas bacteria [2]. Based on the gross lesions, particularly in the omphalitis and yolk sac infections, the agent may invade the birds throughout the navel, and spread systemically through the yolk sac. Actually, most reports related to *P. aerusinosa* infection in the chicks considered that the most important source of infection was environment contamination [2, 4, 7].

Entry or transmission route is known as an important factor, which influence the virulence of the bacteria. In the present study, the inoculation of the *P. aerusinosa* through the IY route caused higher mortality and lower mean death time than that of IM route for chicks. This is because the first line of defense is eliminated when *P. aerusinosa* is inoculated in the yolk, thus the agent multiply in the yolk and enter the blood stream directly and invade all organs rapidly. This result was similar to the results of previously reports [7].

On the basis of the characteristic clinical signs in the field, the pathologic findings, and the identity of the isolates, the disease that occurred in the broilers was finally diagnosed as *P. aerusinosa* infection and was confirmed as the first report in broilers in Korea.

In order to control the disease, further studies will be needed to be conducted detect and monitor the Pseudomonas infection outbreak in broilers.

References

1. **Bapat JA, Kulkarni VB, Nimje DV.** Mortality in chicks due to *Pseudomonas aeruginosa*. Indian J Anim Sci 1985, **55**, 538-539.
2. **Barnes HJ.** Miscellaneous Bacterial Diseases. In: Diseases of Poultry. 11th ed. pp. 852-854, Iowa State University Press, Ames, 2003.
3. **Carter GR.** Essentials of Veterinary Bacteriology and Mycology. 1st ed. pp. 210-212, Michigan State University Press, East Lansing, 1982.
4. **Devriese LA, Viaene NJ, Medts GDE.** Pseudomonas

aerusinosa infection on a broiler farm. Avian Pathol 1975, **4,** 233-237.

5. **Joklik WK, Willett HP, Armos DB, Wilfert CM.** Zinsser Microbiology. 19th ed. pp. 487-492. Appleton-Century-Crofts, New York, 1988.
6. **Harry EG.** The effect on embryonic and chick mortality of yolk contamination with bacteria from the hen. Vet Rec 1957, **69**, 1433-1439.
7. **Walker SE, Sander JE, Cline JL, Helton JS.** Characterization of Pseudomonas aerusinosa isolates associated with mortality in broiler chicks. Avian Dis 2002, **46**, 1045-1052.