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CASE REPORT

Clinical and Morphological Studies on Spontaneous Cases of *Pseudomonas aeruginosa* Infections in Birds

I Dinev[1], S Denev[2]* and G Beev[2]

[1]Department of General and Clinical Pathology, Faculty of Veterinary Medicine; [2]Department of Biochemistry and Microbiology, Faculty of Agriculture, Trakia University, 6000 Stara Zagora, Bulgaria
*Corresponding Author: stefandenev@hotmail.com

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ABSTRACT

Clinical, pathoanatomical, histological, and bacteriological studies were performed on broiler chickens, growing broiler parents, and growing egg layers, in three different poultry farms, after an outbreak of *Pseudomonas aeruginosa* infections. The method of contamination of the birds was established. Several local and systemic clinico-morphological forms of spontaneous *P. aeruginosa* infections in various categories of stock birds were described: cases of *P. aeruginosa* infection resulting from injection of contaminated vaccines; case of *P. aeruginosa* infections through contaminated aerosol vaccine and cases of pododermatitis, periarthritis and arthritis in broiler chickens associated with *P. aeruginosa* infection. In different cases mortality range between 0.5 and 50%. The results showed that apart from embryonic mortality in hatcheries, and septicemic infections in newly hatched chickens, the pathogenicity of *P. aeruginosa* was associated with localized and systemic lesions in this category, as well as in young and growing birds. On one hand, these results have a theoretical significance, contributing for the confirmation and expansion of the wide array of clinico-morphological forms of *P. aeruginosa* infections in birds. On the other hand, the knowledge on these forms has a purely practical significance in the diagnostics of *P. aeruginosa* infections by poultry pathologists and veterinary practitioners.

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INTRODUCTION

Pseudomonas aeruginosa is the representative of the genus with the highest importance in avian pathology (Fodor, 2007). It is ubiquitous, often associated with soil, drinking water, and humid environments (Mohamed, 2004; Mena and Gerba, 2009). In general, studies on *P. aeruginosa* infection in poultry during the last decade are sparse. Basically, the investigations on *P. aeruginosa* infections in birds aimed at studying the morphological, cultural, and biochemical traits, as well as at testing the antimicrobial sensitivity of the obtained isolates (Walker *et al*., 2002; Ogunleye, 2012). A small number of reports show that the pathogenicity of *P. aeruginosa* in birds is mainly related with septicemic and respiratory infections, sinusitis, keratitis, keratoconjunctivitis, and embryonic death rates in hatcheries (Hartl *et al*., 1997; Hussein *et al*., 2008; Hai-ping, 2009). In the current literature, to our best knowledge, we could not find any up to date reports about *P. aeruginosa* infection of broiler chickens and layers. In addition, it is the first clinical case on the problem in Bulgaria.

The aim of the current study was to present some clinical and morphological manifestations of spontaneous cases of *Pseudomonas aeruginosa* infections in various categories of stock birds.

CLINICAL EXAMINATIONS

Clinical and pathoanatomical studies were performed on broiler chickens, growing broiler parents, and growing stock egg layers, in three different poultry farms, after outbreaks of spontaneous *P. aeruginosa* infections. Samples from internal organs (heart, liver, spleen, and bone marrow), subcutaneous tissue, and content of inflamed joints were taken for bacteriological and histopathological studies. The respective samples were tested through the standard bacteriological and histological methods. Bacterial isolates were identified by the API 20 NE identification system (BioMérieux®, France).

CASES PRESENTATION

***Pseudomonas aeruginosa* infections resulting from contaminated vaccines:** In this case, 21 000 stock egg-layers (Hy-line) were hatched in a commercial hatchery, where they were vaccinated against the Marek's disease with a commercial HVT vaccine. For half of the chickens, the vaccine was administered by an automated electrical vaccinator in the neck, while for the others–via automated syringe, in the left thigh region. The chickens were sold to a farm specialized in the breeding of growing stock egg layers, and kept under normal conditions. The occurrence of neural signs started about 24 h after vaccination. These were lack of coordination, ataxia, prostration, marked limping with the left leg and high death rates. In 72-96 h after vaccination, mortality had reached approximately 50%. No pathoanatomical changes were found in the chickens, which died early. A few hours afterwards, gross lesions begun to appear. In some of the chickens, these were exhibited through diffuse subcutaneous edemas in the area of the neck, sometimes spreading over the head too. The covering skin in some chickens was macerated, and the feathers – fallen or easily detached together with the surface epidermal layer (Fig. 1 and 2). Subcutaneously, edemas with serous, sometimes even hemorrhagic exudate were found (Fig. 3 and 4). The lesions in the subcutaneous area of the left thigh were similar. Subcutaneous hemorrhages were found in the breast muscles of some chickens. Hyperemia, subcapsular hemorrhages and dystrophy were observed in the liver. Histologically, inflammatory hyperemia and dystrophic necrobiotic changes in both liver and heart parenchyma were observed.

***Pseudomonas aeruginosa* infections through contaminated aerosol vaccine:** In this case, 6 000 growing broiler parents at the age of 56 days were given a commercial aerosol vaccine. The first clinical signs appeared about a week later, manifested by periorbital edemas, lack of coordination, and retarded growth. In a part of the chickens, conjunctivitis and dense fibrous plaques in the medial eye corners were observed (Fig. 5). The neural signs were clinically exhibited by opisthotonus and torticollis (stiff neck). Pathoanatomically, inflammatory and necrotic changes were found in the frontal skull bones. A week after the appearance of the first clinical signs, mortality rate was approximately 4%. In some of the dead chickens, the necropsy showed a diffuse fibrinous polyserositis. The deposited fibrinous pseudomembranes had a specific amber color. Histologically, serofibrinous pericarditis and perihepatitis were found in the latter.

Pododermatitis, periarthritis and arthritis in broiler chickens caused by *Pseudomonas aeruginosa* infection: Two separate batches of 20 000 broilers each, bred by routine technology on straw bedding, in conditions of increased humidity were investigated. In the first case, lameness appeared by the end of the first week. Clinically, edemas of a various degree were observed on the plantar surface of the hind limbs or tibiotarsal joints. The edematous surface was warm and reddened. A thick, cream-like exudate was present during incision (Fig. 6).

Fig. 1: Diffuse subcutaneous oedemas spread over the neck and head of chicken, 24 h after vaccination with *P. aeruginosa*-contaminated Marek's vaccine; **Fig. 2:** Covering skin is often macerated, and the feathers fall or are easily separated, together with the surface epidermal layer in chicken suffering from *P. Aeruginosa* infection.

Fig. 3: Subcutaneous serous inflammatory edema in the neck area; **Fig. 4:** Subcutaneous hemorrhagic inflammatory edema in the neck area in chicken suffering from *P. Aeruginosa* infection.

Fig. 5: Conjunctivitis, with consequent keratitis and panophthalmitis as an outcome of a local form of *P. aeruginosa* infection in a growing broiler parent, eight weeks of age; **Fig. 6:** Pododermatitis (foot inflammation) of the right lower limb in a broiler, seven days of age, caused by a *P. aeruginosa* infection; **Fig. 7:** Periarthritis in the area of the tibiotarsal joint in a broiler chicken, caused by a *P. aeruginosa* infection, caused by bad environment conditions.

The morbidity rate reached approximately 10%, while mortality rate - 0.5%.

In the second case, the broiler chickens at the age of 5 months exhibited acute uni- or bilateral lameness, lying down, incapability to move, and sometimes death from exhaustion. In lame chickens, edemas and increased temperature of the tibiotarsal and knee joints were found (Fig. 7). Thick, cream-like exudate with a cream color came out during incision. In some of the dead chickens, a serofibrinous pericarditis was found. In this case,

morbidity rate reached approximately 15% and mortality rate-10%.

Throughout the bacteriological tests, in all described cases, *P. aeruginosa* was isolated and identified in pure culture from internal organs, as well as from subcutaneous tissue and joint exudate in the cases with arthritis. In the first two cases of post-vaccination infection, *P. aeruginosa* was isolated and identified also in pure culture in the residue of dissolved vaccine.

DISCUSSION

The results from our studies showed that apart from embryonic mortality in hatcheries, and septicemic infections in newly hatched chickens, the pathogenicity of *P. aeruginosa* was associated with localized and systemic lesions in this category, as well as in young and growing birds. The results we obtained complemented the knowledge on some of the already known clinical and morphological forms of *P. aeruginosa* infections in birds (Walker *et al*., 2002). On one hand, these results have a theoretical significance, contributing for the confirmation and expansion of the wide array of clinico-morphological forms of *P. aeruginosa* infections in birds (Lin *et al*., 1993). On the other hand, the knowledge on these forms has a purely practical significance in the diagnostics of *P. aeruginosa* infections by poultry pathologists and veterinary practitioners.

We could assume (without confirming it experimentally), that the localized or septicemic forms of *P. aeruginosa* infections are dependent on the path of the entrance route of the agent into the body, as well as on the age and resistance of the host animal (Mohamed, 2004). In the spontaneous cases we observed, when the infection entered parenterally (though a Marek's vaccination), mortality in newly hatched chickens reached 50%. The observed arthritis, pododermatites, conjunctivitis, etc. in growing broiler parents and broiler chickens, for which the most probable door of the infection are skin and mucosal injuries (after aerosol vaccination or high humidity rearing conditions) allowed us to assume that these are the paths to localized forms of infection, which also cause significant losses within the flocks (Fodor, 2007). The clinically observed neural symptoms in some of the chickens infected with *P. aeruginosa* could be explained with the discovered lesions in the frontal skull bones, which most probably appeared through the spreading of the inflammation process from adjacent soft tissues.

The sources of infection (whether by hatchery conditions, vaccination facilities, infected embryos, the environment, or other) in our cases, was not clearly defined. Yet, according to us, it seemed that one of the most significant routes of infection spreading was via a parenteral route through contaminated vaccines, with insufficient hygiene, which led to significant losses.

The results of the current study provide several important implications. This indicates that *P. aeruginosa* infection may be one of the major problems of broiler chickens and layers. Presenting this clinical case would be beneficial, because there is scarce literature on the problem, and it is the first study that presents some clinical and morphological manifestations of spontaneous cases of *P. aeruginosa* infections in various categories of stock birds in Bulgaria.

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