Praca oryginalna *Original paper*

Isolation of Gallibacterium spp. from peacocks with respiratory tract infections

MAGDALENA RZEWUSKA, EWA KARPIŃSKA*, PIOTR SZELESZCZUK*, MARIAN BINEK

Division of Bacteriology and Molecular Biology, Department of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Ciszewskiego 8, 02-776 Warsaw, Poland
*Division of Avian Diseases, Department of Clinical Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Ciszewskiego 8, 02-776 Warsaw, Poland

Rzewuska M., Karpińska E., Szeleszczuk P., Binek M.
Isolation of Gallibacterium spp. from peacocks with respiratory tract infections

Summary

Gallibacterium spp. is a new genus of the family Pasteurellaceae. These Gram-negative rods are a part of the physiological flora of the upper respiratory tract, genital tract and gastrointestinal tract of birds and have been found in different species of domestic and wild birds. They are opportunistic pathogens that were isolated from birds with pathological lesions or conditions. This paper presents the first case of Gallibacterium anatis isolation from peacocks with respiratory tract infections. Three isolates from lung tissue were identified on the basis of phenotypic properties as Gallibacterium anatis biovar Haemolytica. The whole flock of peacocks was isolated in a small closed farm building for 5 weeks. During this period the first clinical signs of the disease were observed. That situation could result in immunosuppression in birds, which is one of the predisposing factors of opportunistic G. anatis infections. This observation confirms the important role of the stress factor in an infection caused by these bacteria.

Keywords: Gallibacterium anatis, peacocks, respiratory tract

Bacteria belonging to *Gallibacterium* species are Gram-negative, facultatively anaerobic, non-motile rods. *Gallibacterium* is a new genus of the family *Pasteurellaceae*, proposed by Christensen et al. in 2003 for bacteria previously classified as „*Actinobacillus salpingitidis*", *Pasteurella haemolytica*-like, or *Pasteurella anatis* (8, 12, 14). These bacteria were reclassified on the basis of the sequence of 16S rRNA genes and an analysis of DNA homology by DNA-DNA hybridization (7, 8). The genus *Gallibacterium* is a heterogeneous group of bacteria characterized by a high level of phenotypic diversity. According to Christensen et al. (8) this genus includes only one species *G. anatis*, but they proposed distinguishing two biovars (*G. anatis* bv. Haemolytica and *G. anatis* bv. Anatis) and two *Gallibacterium* genomospecies.

It has been suggested that *Gallibacterium* spp. are opportunistic pathogens. They are a part of the physiological flora of the upper respiratory tract, genital tract and gastrointestinal tract of birds (2). These bacteria were found in different species of domestic and wild birds, like hens, turkeys, ducks, geese, pheasants, partridges, parrots (3, 13). However, *Gallibacterium* were also isolated from birds with pathological lesions or conditions, such as respiratory tract lesions, septicemia, salpingitis, peritonitis (4, 11, 15, 16). In this paper we would like to report the first case of isolation of *Gallibacterium anatis* from peacocks (*Pavo cristatus*) with infections of the respiratory tract.

Material and methods

Clinical material. Lung and liver samples were collected for bacteriological examination. The specimens were obtained from three peacocks from a flock numbering over 60 birds. The clinical signs such as dyspnea, mucopurulent discharge from nasal cavities, conjunctivitis and oedema of the head soft tissues were observed. Due to uni- or bilateral oedema, the most frequent presentation of the disease was deformation in the region of orbits and infraorbital sinuses. Birds were lethargic and their water and feed intake decreased.

Culture conditions. The specimens were streaked on Columbia agar supplemented with 5% sheep blood (bioMérieux, France) and MacConkey agar (bioMérieux, France). The inoculated plates were incubated at 37°C for 48 hours in microaerophilic conditions.

Phenotypic characterization. Phenotypical characters of isolates included colony morphology on a medium containing blood, cell morphology observed in Gram-staining slides and biochemical properties. Biochemical activity of isolated bacteria were determined using API 20 E, API STAPH and API ZYM tests (bioMérieux, France) according to the manufactures instructions. Additionally, pro-

duction of catalase (3% H_2O_2) and oxidase (bioMérieux, France) were tested.

Antimicrobial susceptibility. Isolates were tested for antibiotic susceptibility using the standard disk diffusion method. The test was performed on Mueller Hinton Agar with 5% sheep blood (bioMérieux, France) and disks containing specified amounts of antibiotics (bioMérieux, France; Mast Diagnostics, UK) were used. The plates were incubated at 37°C for 24 hours in microaerophilic conditions.

Results and discussion

In the lungs of all autopsied birds purulent foci with a size from wheat seed up to lentil seed were observed (fig. 1). Lung tissue was congested and around the foci the margin line of congestion was clearly visible. The liver was fragile and spleen mildly enlarged. In direct microscopic slides from lung and liver samples Gram-negative rods were detected. Numerous, greyish-yellowish, beta-haemolytic colonies were isolated on Columbia agar from all examined lung tissues (fig. 2). Growth of isolates on MacConkey agar was poor. In the microscopic examination of these colonies Gram-negative small rods were observed. Results of biochemical tests for three isolated strains were identical and were positive in the following reactions: catalase, oxidase, ONPG (beta-galactosidase), alpha-glucosidase, VP (Voges-Proskauer), alkaline phosphatase and acidification without gas for D-glucose, D-fructose, D-mannose, D-mannitol, D-sucrose, D-lactose, D-trehalose, D-xylose, D-sorbitol and inositol. The negative reactions were obtained for: beta-glucuronidase, indol, urease, gelatinase, reduction of nitrates and acidification without gas for L-arabinose, D-maltose, D-raffinose. On the basis of phenotypic characters these isolates were identified as *Gallibacterium anatis* biovar Haemolytica according to Christensen et al. (8). These Gram-negative rods were found only in the lung specimens, but not in the liver tissue.

The isolated strains of *Gallibacterium* were susceptible to amoxicillin, amoxicillin/clavulanic acid, streptomycin, neomycin, gentamycin, tetracycline, cefuroxime, florphenicol, enrofloxacin, flumequine, and were resistant to difloxacin.

Gallibacterium anatis biovar Haemolytica shows phenotypic similarity to *Mannheimia haemolytica*. The most important phenotypic differences between these bacteria were production of alpha-glucosidase and acidification of D-mannose without gas. These reactions are positive for *G. anatis*, but negative for *M. haemolytica* (1).

The pathogenicity of *Gallibacterium* is poorly understood. There is limited data on the virulence factors of these bacteria. In some earlier studies *Gallibacterium* strains that differed in their virulence level had been described (2, 13). However, no correlation between virulence and phenotypic characterization of these strains was observed. Probably the recently reported metalloproteases that degrade chicken IgG, could play an important role in the mechanisms of infections caused by *Gallibacterium* (9). It also seems that the immune status of birds has a crucial effect on the pathogenesis of infections. The results of the experimental study in normal and immunosuppressed chickens infected by *Gallibacterium* indicated that the course of the disease in immunosuppressed birds is acute, with a high mortality rate (6).

In the reported case, peacocks were exposed to stress caused by a prolonged stay in an enclosed space. The whole flock of peacocks had been isolated in a small farm building, not settled earlier by any animal species, due to legal restrictions after the avian influenza

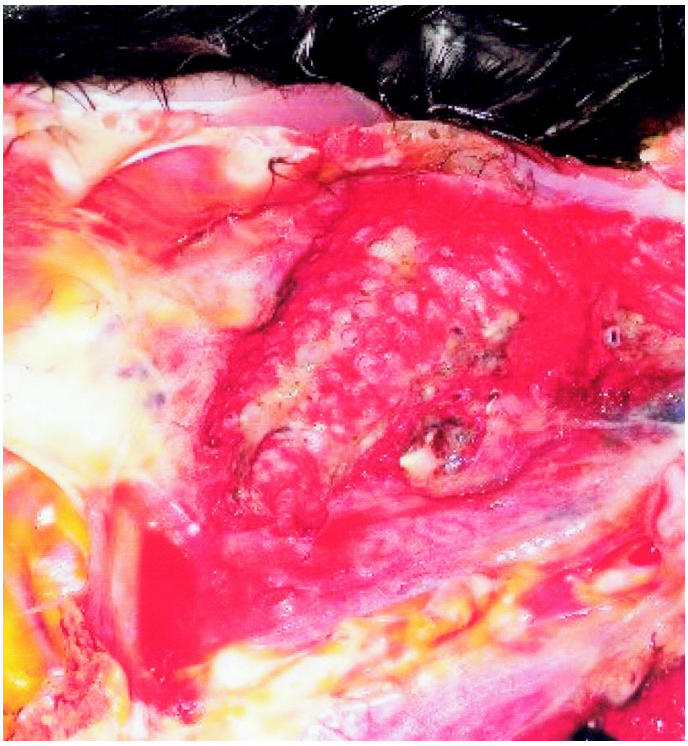


Fig. 1. Focal purulent lesions in the lungs of a peacock with *Gallibacterium anatis* infection

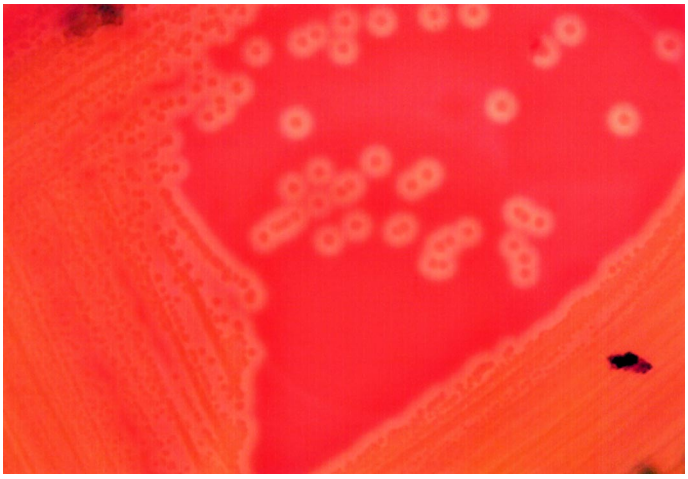


Fig. 2. Colonies of *Gallibacterium anatis* biovar Haemolytica on Columbia Agar with sheep blood after 24 hours' incubation

virus detection in wild-fowl in Poland at the beginning of 2006. Birds were strictly enclosed for about 5 weeks and during this period the first cases of disease and first losses were observed. During the period of isolation the number of birds with clinical signs of the disease gradually increased and dyspnea was more severe. A total of ten peacocks died. On autopsy the predominant sign in all dead birds was inflammatory lesions in the respiratory system. Mucosa of the lower portion of the trachea was congested and thick with a large amount of mucopurulent discharge. The air sacs were mildly thickened.

These conditions probably led to immunosuppression in the birds, which is one of the predisposing factors of opportunistic *G. anatis* infections. The important role of the stress factor was confirmed by the observation that the state of health of the birds improved after releasing them on the flow-run.

In the case of *G. anatis* infections the lesions induced are not pathognomonic, and isolation of the aetiological agent is very important. *Gallibacterium anatis* like most of the genus of *Pasteurellaceae* are phenotypically a heterogeneous group of bacteria and their identification by classical methods can be difficult. It seems that genotypic diagnostic techniques may allow for a better understanding of the pathogenesis and epidemiology of *Gallibacterium* infections (5, 10).

References

1. *Angen O., Mutters R., Caugant D. A., Olsen J. E., Bisgaard M.*: Taxonomic relationships of the (Pasteurella) haemolytica complex as evaluated by DNA--DNA hybridizations and 16S rRNA sequencing with proposal of Mannheimia haemolytica gen. nov., comb. nov., Mannheimia granulomatis comb. nov., Mannheimia glucosida sp. nov., Mannheimia ruminalis sp. nov. and Mannheimia varigena sp. nov. Int. J. Syst. Bacteriol. 1999, 49, 67-86.
2. *Bisgaard M.*: Incidence of Pasteurella haemolytica in the respiratory tract of apparently healthy chickens and chickens with infectious bronchitis. Characterisation of 213 strains. Avian Pathol. 1977, 6, 285-292.
3. *Bisgaard M.*: Ecology and significance of Pasteurellaceae in animals. Zentbl. Bakteriol. 1993, 279, 7-26.
4. *Bisgaard M., Dam A.*: Salpingitis in poultry. II. Prevalence, bacteriology, and possible pathogenesis in egg-laying chickens. Nord. Vetmed. 1981, 33, 81-89.
5. *Bojesen A. M., Christensen H., Nielsen O. L., Olsen J. E., Bisgaard M.*: Detection of Gallibacterium spp. in chickens by fluorescent 16S rRNA in situ hybridization. J. Clin. Microbiol. 2003, 41, 5167-5172.
6. *Bojesen A. M., Nielsen O. L., Christensen P. J., Bisgaard M.*: In vivo studies of Gallibacterium anatis infection in chickens. Avian Pathol. 2004, 33, 147--154.
7. *Bojesen A. M., Torpdahl M., Christensen H., Olsen J. E., Bisgaard M.*: Genetic diversity of Gallibacterium anatis isolates from different chicken flocks. J. Clin. Microbiol. 2003, 41, 2737-2740.
8. *Christensen H., Bisgaard M., Bojsen A. M., Mutters R., Olsen J. E.*: Genetic relationships among avian isolates classified as Pasteurella heamolytica, „Actinobacillus salpingitidis" or Pasteurella anatis with proposal of Gallibacterium anatis gen. nov., comb. nov. and description of additional genomospecies within Gallibacterium gen. nov. Int. J. Syst. Evol. Microbiol. 2003, 53, 275-287.
9. *Garcia-Gomez E., Vaca S., Perez-Mendez A., Ibarra-Caballero J., Perez--Marquez V., Tenerio V. R., Negrete-Abascal E.*: Gallibacterium anatis – secreted metalloproteases degrade chicken IgG. Avian Pathol. 2005, 34, 426--429.
10. *Gautier A. L., Dubois D., Escand F., Avril J. L., Trieu-Cuot P., Gaillot O.*: Rapid and accurate identification of human isolates of Pasteurella and related species by sequencing the sodA gene. J. Clin. Microbiol. 2005, 43, 2307--2314.
11. *Mirle C., Schongarten M., Meinhart H., Olm U.*: Studies into the incidence of Pasteurella haemolytica infections and their relevance to hens, with particular reference to diseases of the egg-laying apparatus. Monatsh. Vetmed. 1991, 45, 545-549.
12. *Mraz O., Vladik P., Bohacek J.*: Actinobacilli in domestic fowl. Zentbl. Bakteriol. Hyg. I. Abt. Orig. A 1976, 236, 294-307.
13. *Mushin R., Weisman Y., Singer N.*: Pasteurella haemolytica found in respiratory tract of fowl. Avian Dis. 1980, 24, 162-168.
14. *Mutters R., Ihm P., Pohl S., Frederiksen W., Mannheim W.*: Reclassification of the genus Pasteurella Trevisan 1887 on the basis of deoxyribonucliec acid homology, with proposals for the new species Pasteurella dagmatis, Pasteurella canis, Pasteurella stomatis, Pasteurella anatis, and Pasteurella langaa. Int. J. Syst. Bacteriol. 1985, 35, 309-322.
15. *Shaw D. P., Cook D. B., Maheswaran A. K., Lindeman C. J., Halvorsond A.*: Pasteurella haemolytica as a co-pathogen in pullets and laying hens. Avian Dis. 1990, 34, 1005-1008.
16. *Suzuki T., Ikeda A., Shimada J., Yanagawa Y., Nakazawa M., Sawada T.*: Isolation of „Actinobacillus salpingitidis"/avian Pasteurella haemolytica-like isolate from diseased chickens. J. Jpn. Vet. Med. Assoc. 1996, 49, 800-804.

Autor's address: PhD Magdalena Rzewuska, Department of Preclinical Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Ciszewskiego str. 8, 02-776 Warsaw, Poland; e-mail: rzewuskam@tlen.pl