Summary

Introduction. Infection with strains of *Erysipelothrix rhusiopathiae* has been a problem in waterfowl farming in Poland for many years. The disease induced by these bacteria, known as erysipelas, occurs mainly in flocks of geese, and less often in ducks. The infection takes the form of sepsis with vascular and organ lesions (Bobrek et al. 2016). It causes considerable economic losses associated with deaths and treatment of birds, and in the case of breeding flocks, also due to declines in laying rates. Our own observations indicate that the disease most often affects birds after rearing, and that the pathogen is difficult to eliminate from the flock. Antibiotic treatment is usually effective, but after it is terminated recurrence of the disease is common. Currently no erysipelas vaccine for poultry is authorized for marketing in Poland. For this reason, some veterinarians attempt to protect the flock by using vaccines registered for use in pigs. However, field reports indicate that these measures do not always provide effective immunization against infection.

E. rhusiopathiae bacteria are Gram-positive rods with high growth requirements. They cause infection in mammals, including humans, birds, and fish, and their main reservoir is considered to be pigs. Literature reports on erysipelas in pigs are currently the main source of knowledge about the serotypes, drug susceptibility and virulence factors of *E. rhusiopathiae* strains. This bacterial species includes 17 serotypes. Strains infecting pigs usually belong to serotypes 1a, 1b and 2. Virulence factors of *E. rhusiopathiae* include proteins facilitating infection of host tissues and factors determining resistance to phagocytosis and complement. One of the best-known virulence factors is surface protein Spa, against which an immune response is generated in the host. The occurrence of erysipelas in waterfowl has been reported only in a few case studies (Bobrek et al. 2016, Bobrek and Gaweł 2015, Janowska et al. 1978).

The main **aim of the study** was the serotypic characterization and determination of antibiotic susceptibility, genotypic profiles of resistance and virulence as well as variants of the antigenic SpaA protein of *E. rhusiopathiae* strains from clinical cases of erysipelas in geese and ducks raised in Poland. The work also assumes the analysis of anatomopathological changes and the determination of the distribution of erysipelas cases in age groups of birds and periods of calendar year.

Material and methods. The starting material for the research was dead geese and ducks delivered to the Vet-Lab Brudzew Dr Piotr Kwieciński veterinary laboratory for diagnostic purposes during the period from January 2019 to December 2021. The clinical

data collected were used to determine the distribution of erysipelas cases in birds in several age groups and periods of the calendar year. Anatomopathological examination of the dead birds was performed to assess lesions in the internal organs, skin, subcutaneous tissue, and joints. *E. rhusiopathiae* strains from the tissues of dead birds were identified using commercial real-time PCR tests, MALDI-TOF mass spectrometry, and the VITEK® 2 GP cards with a biochemical panel. The drug susceptibility of *E. rhusiopathiae* isolates (n=60) was determined by the microdilution method in broth, and genotypic resistance and virulence profiles and serotypes were determined by PCR. The gene *spaA* encoding the immunogenic protein SpaA was sequenced, and the DNA sequence was translated to the protein sequence. Based on the analysis of the obtained protein sequences, the SpaA variants found in field isolates and the homology between the field isolates and the vaccine strain *E. rhusiopathiae* R32E11 were determined. The final stage of the research was genotyping of *E. rhusiopathiae* strains, using rep-PCR and RAPD.

Results. A number of significant results were obtained. The most important of these are listed below.

- The distribution of erysipelas cases in age groups of geese and periods of the calendar year differed significantly from the assumed even distribution; the most cases were noted in August and September in the age group between 11 and 13 weeks.
- Necropsy of birds infected with *E. rhusiopathiae* showed anatomopathological lesions characteristic of generalized infection, affecting multiple organs.
- All isolates tested were identified as *E. rhusiopathiae*; the results of the three identification methods used (named above) were in full agreement. In the case of the method using VITEK® 2 GP cards, repeatability in terms of the percentage probability of correct identification and analysis time was limited.
- The *E. rhusiopathiae* isolates (n = 60) belonged to seven serotypes, among which serotypes 5 (38.3%), 1b (28.3%) and 8 (15%) predominated.
- All *E. rhusiopathiae* isolates were susceptible to β-lactam antibiotics and florfenicol. The vast majority of isolates were resistant to tetracycline (85%) and enrofloxacin (80%). The percentages of strains resistant to the remaining antimicrobials ranged from 3.3% to 16.7%. Ten strains (16.7%) were found to be multi-drug resistant.
- The genotypic drug-resistance profiles of the *E. rhusiopathiae* strains (n = 60) corresponded to their phenotypic resistance. The gene *tetM* was detected in all tetracycline-resistant strains. The gene *lnuB* was present in 10 of 11 lincosamide-resistant strains, and *lsaE* was detected in all isolates resistant to tiamulin. The gene *ermB* was detected in one strain resistant to macrolides and lincosamides. The gene *ant(6)-Ia* was

present in four isolates with high MICs for streptomycin and spectinomycin. In enrofloxacin-resistant strains, there was a mutation in the *gyrA* gene.

- There was very little variation between the genotypic virulence profiles of the *E. rhusiopathiae* isolates. The gene *spaA* encoding the immunogenic protein SpaA was detected in 59 strains, while only one isolate contained the *spaB* gene. From 98.3% to 100% of strains possessed the *nanH.1* and *nanH.2* genes coding for neuraminidase and other genes whose products could facilitate *E. rhusiopathiae* infection of host tissues, i.e. *sub*, *hlyA*, *fbpA*, ERH_1356, *intI*, *hlyIII*, *rspA* and *rspB*, or determine the resistance of bacterial cells to phagocytosis and complement activation – *cpsA* and *algI*.
- Based on the nonsynonymous mutations detected in the *spaA* sequences of the isolates, 9 variants of the SpaA protein were distinguished. The sequences of the SpaA immunoprotective domain (antigenic) of all field strains differed from the sequence of the vaccine strain R32E11, and in the vast majority of isolates (91.5%) there were differences in 7, 8 or 9 amino acids.
- The rep-PCR genotyping method showed that the use of the primer $(GTG)_5$ makes it possible to distinguish *spaA*-positive and *spaB*-positive strains of *E. rhusiopathiae*, but does not enable differentiation among *spaA*-positive isolates. In the case of RAPD, the best intraspecific differentiation results were obtained using the primer M13.

Conclusions. The results obtained during the research for the doctoral thesis, pertaining to characteristics of *E. rhusiopathiae* strains, the occurrence of erysipelas in waterfowl, and pathological legions in the course of the disease, provide valuable new information contributing to knowledge in the areas of poultry diseases, veterinary microbiology, and epidemiology, and are also important for veterinary practice, both in diagnosis and treatment of erysipelas. Data on the aetiological agent of erysipelas can be used to develop effective strategies for controlling infections in waterfowl.