Occurrence of *Enterococcus* spp. in poultry in Poland based on 2014-2015 data

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Summary

Bacteria of the genus *Enterococcus* are mainly commensals building natural microflora in the digestive tract of birds and mammals. They belong to the potentially pathogenic microorganisms. Among poultry, infections caused by enterococci were reported in chickens, turkeys, ducks and ostriches. The aim of this study was to evaluate the occurrence of enterococci in poultry in Poland, including identification of enterococcus species composition and determination of the age of birds. The analysis was based on data obtained from 2014-2015 from Division of Avian Diseases at Warsaw University of Life Sciences-SGGW and four veterinary laboratories in Poland: Lab-Vet, Tarnowo Podgórne; SLW Biolab, Ostróda; Vetdiagnostica, Solec Kujawski; Vet-Lab Brudzew. Seven enterococcal species were isolated from broiler chickens (CB), commercial layers (CL), and broiler breeder flocks (BB), nine from all poultry types (chickens, turkey, ducks and geese). The most often isolated enterococci from CB were *E. faecalis* (57%) > *E. cecorum* (7%) > *E. faecium* (5.2%) > *E. hirae* (3.6%) > *E. gallinarum* (2.5%) > *E. casseliflavus* (0.7%) > *E. durans* (0.2%). Seven *Enterococcus* species were isolated from sources associated with poultry, most often *E. faecalis* > *E. faecium* > *E. cecorum* > *E. hirae*. The differences in the occurrence of particular enterococcal species were observed between CB, BB and CL. The mean age at the time of isolation of *E. cecorum* was approx.: 3.6 weeks in CB, 27.5 weeks in BB, 33.3 weeks in CL, 12.9 weeks in turkeys, 3.6 weeks in ducks, 39.5 weeks in geese. *E. faecalis* and *E. faecium* dominated in samples obtained from hatching eggs, dead-in-shell embryos and from samples related to poultry environment.

In conclusion, this study indicates the high prevalence of bacteria of the *Enterococcus* genus in poultry. The present findings demonstrate the differences in *Enterococcus* species between poultry groups, including with regard to age. In total 10 enterococcal species (*E. faecalis*, *E. cecorum*, *E. hirae*, *E. faecium*, *E. gallinarum*, *E. casseliflavus*, *E. durans*, *E. avium*, *E. thailandicus*, *E. aquimarinus*) were detected in poultry, poultry environmental samples, hatching eggs and dead-in-shell embryos. *Enterococcus faecalis* and *E. cecorum* were found in all above-mentioned sources.

Keywords: *Enterococcus* species, poultry, enterococci

Bacteria of the *Enterococcus* genus constitute the natural intestinal microflora of mammals and birds, and are ubiquitous in the environment. Until today, 53 *Enterococcus* species and 2 subspecies have been recognized. In the last 6 years (2010-2016) 13 species and 2 subspecies were described. Among all known enterococci, only a few species are isolated from birds, mainly: *E. faecalis*, *E. faecium*, *E. hirae*, *E. cecorum*, *E. durans*, *E. avium*, *E. casseliflavus*, *E. gallinarum*,

E. raffinosus and *E. columbae* (from pigeons), *E. alcedinis* (from common kingfishers). The composition of the enterococcal intestinal flora varies with the age of birds. Moreover, changes in the enterococcal species composition are observed. In 1-day-old chicks *E. faecalis* and *E. faecium* were found mainly in intestines, while *E. durans* in the crop. According to Devriese et al. (5) the earliest occurrence of *E. cecorum* in the digestive tract of chickens was recorded at the age of

3-4 weeks. *Enterococcus cecorum* was predominant enterococcal component in the crop and intestines in chickens by 12 weeks of age. However, *Enterococcus durans* could be found in the crop of 1-day-old chicks, later (at an age of 3-4 weeks) in the intestines (5). Enterococci are potentially pathogenic bacteria with the ability to cause serious diseases in birds. So far, infections caused by *Enterococcus* spp. have been described in chickens, turkeys, ducks, ostriches, pigeons and canaries. Among 53 enterococcal species, *E. faecalis*, *E. cecorum*, *E. hirae*, *E. faecium* and *E. durans* have the most crucial role in avian pathology. *Enterococcus faecalis* has been described as a cause of omphalitis, endocarditis, meningitis, fibrinous arthritis and/or tenosynovitis. Moreover, *E. faecalis* may participate in the etiology of Pulmonary Hypertension Syndrome (PHS) in broilers (17). Investigations by Landman et al. (11) confirmed that arthropathic and amyloidogenic *E. faecalis* strains have the potential to induce amyloid arthropathy and chronic infections in brown layers. *Enterococcus faecalis* has also been shown to be involved in First-Week Mortality Syndrome (FWM) in layers (14). However, *E. cecorum* may cause enterococcal spondylitis, femoral head necrosis, arthritis. Infection with *E. cecorum* can constitute a serious health problem, usually for commercial broiler chickens and broiler breeder flocks (2, 7, 12). Recently, *E. cecorum*-associated disease was reported in Pekin ducks and in racing pigeons (9). Other species such as *E. hirae* and *E. durans* may cause endocarditis and encephalomalacia in chickens. There are differences in age-related susceptibility to strains causing endocarditis. Septicaemia and endocarditis caused by *E. durans* seem to be limited to mature birds, whereas infections with *E. hirae* appear to include young chickens (3, 18). *Enterococcus*-associated encephalomalacia has been noted in 1-week-old to 2-week-old broilers and commercial layers (1, 3, 4). In the literature there is still not enough information which enterococcal species are associated with certain birds.

The aim of this study was to evaluate the prevalence of *Enterococcus* spp. in poultry in Poland with regard to species composition.

Material and methods

The analysis is based on data from 01.01.2014-01.07.2015 collected in Poland from the following veterinary laboratories: Division of Avian Diseases, Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, WULS-SGGW, Warsaw; Lab-Vet, Tarnowo Podgórze (Greater Poland); SLW Biolab, Ostróda (Warmia-Masuria); Vetdiagnostica, Solec Kujawski (Kuyavia-Pomerania); Vet-Lab Brudzew (Greater Poland). Materials mainly included samples from affected birds (tissues, swabs, dead-in-shell embryos) collected during standard diagnostic necropsies and from samples related to poultry environment (e.g. feed, water, control swabs from surfaces, chick transport papers with meconium, fluff from hatchery cham-

bers). Bacteriological tests and identification methods were carried out in accordance with the diagnostic procedures applied in individual laboratories. The samples were directly plated onto blood agar containing 5% sterile bovine or sheep blood, or CNA agar with 5% sheep blood, and onto agar containing esculin (Enterococcosel Agar or KAA agar). All agar plates were incubated microaerobically at 37°C for 24 h after which culture results were evaluated based upon colony morphology. Species identification was based on biochemical tests (API rapid ID 32 STREP, bioMérieux, France), or MALDI-TOF and PCR (8).

Results and discussion

During the 2014-2015 period a total of 2828 *Enterococcus* spp. were retrieved from animal sources, from which 82.6% (2337/2828) were retrieved from poultry, 8.8% (250/2828) from a poultry production environment, 2.5% (71/2828) from hatching eggs and dead-in-shell embryos.

The most numerous of enterococcal strains originated from chickens (73.7%; 1723/2337), including 61.2% (1431/2337) from commercial broiler chickens (CB), 7.2% (168/2337) from commercial layers (CL), 3.6% (85/2337) from broiler breeder chickens, and 1.7% (39/2337) from other chickens (no data on the flock type). Among other poultry species, strains originated from turkeys (23.4%; 548/2337), geese (2.1%; 50/2337) and ducks (0.7; 16/2337). Similarly to other studies (15), in our study the most positive samples for enterococci were in broilers. In poultry, 9 of the most frequent identified species were *E. faecalis* (42.5%) > *E. cecorum* (7.9%) > *E. faecium* (4.4%) > *E. hirae* (2.7%) > *E. gallinarum* (2.2%) > *E. casseliflavus* (0.6%) > *E. durans* (0.2%) > *E. avium* (0.16%) > *E. aquimarinus* (0.04%). However, a number of isolates (39.3%) were not recognized to the species level. We found that *E. faecalis* seems to be the dominant enterococcal species in poultry. Our results were consistent with the literature (15). In opposition to other authors, we isolated *E. cecorum* more often, and more rarely *E. faecium*, *E. hirae*, *E. gallinarum*. Data analysis with respect to the specific group of poultry demonstrated the prevalence of 7 different *Enterococcus* species among broiler chickens (CB), commercial layers (CL), and broiler breeders (BB). All mentioned groups were positive for 5 species: *E. faecalis*, *E. cecorum*, *E. hirae*, *E. gallinarum*, *E. casseliflavus*. According to other authors, there are differences in *Enterococcus* species composition between broilers and layers (20), which was also confirmed by our results. In the present study, *E. avium* was not seen in CB, and *E. faecium* in BB, *E. durans* in CL (Fig. 1 A, B, C). The occurrence of enterococcal species in turkeys and waterfowl was shown in Fig. 1 D, E, F. According to Kizerwetter-Swida and Binek (10), enterococci dominate in the caecal flora of 20-day-old embryos and 1-day-old chicks. Some *Enterococcus* species found in 1-day-old chicks may

Fig. 1. A-F Percentage of *Enterococcus* species isolated from different poultry

be absent (or at lower rate) in older chickens (5). Under certain conditions, the presence of *Enterococcus* species non-typical for chicken's age, outside the natural habitat in organisms, may promote infection. Tankson et al. (16) proved that *E. faecalis* may be isolated from the heart and lungs of healthy chickens starting from the 17th day of incubation, and could become pathogenic if predisposing factors prevail. Table 1 shows the mean age of poultry at isolation of *Enterococcus* spp. from diagnostic cases. Olsen et al. (13) showed that 15% of the chicks may be positive for *E. faecalis*

when hatching started, and 70% when sampled 24 h after hatching. We found that the most *E. faecalis* was isolated from 1-day-old chicks (CB 30.4%; BB 62.5%; CL 59.2%; turkeys 34%; geese 33.3%; ducks 57.1%). Out of 7 enterococci identified from hatching eggs, dead-in-shell embryos, *Enterococcus faecalis* and *E. faecium* were recognized more consistently (Fig. 2). Similarly, other authors isolated *E. faecalis* (then *E. faecium*) mainly from broilers between 1 and 5 days of age (15, 19). We found that *E. cecorum* may be seen in all groups of poultry, usually after 3.6 weeks of

Tab. 1. The mean age of poultry at isolation of *Enterococcus* spp. from diagnostic cases

<i>Enterococcus</i> species	Mean age of birds (days/weeks)					
	CB	Chickens BB	CL	Turkeys	Ducks	Geese
<i>E. faecalis</i>	2.1 days	84.4 days (12 weeks)	64.2 days (9 weeks)	28.2 days (4 weeks)	1.4 day	124.3 days (18 weeks)
<i>E. durans</i>	3.3 days	63 days (9 weeks)	ND	59 days (8 weeks)	ND	ND
<i>E. gallinarum</i>	3.4 days	1 day	1 day	19.1 days (3 weeks)	ND	ND
<i>E. hirae</i>	12.3 days	4 days	26.7 days (4 weeks)	42 days (6 weeks)	3 days	ND
<i>E. faecium</i>	3.5 days	ND	154.4 days (22 weeks)	30.2 days (4 weeks)	ND	3 years
<i>E. cecorum</i>	25.4 days (3.6 weeks)	193.6 days (27.5 weeks)	232.8 days (33.3 weeks)	90.1 days (12.9 weeks)	25.1 days (3.6 weeks)	276.3 days (39.5 weeks)

Explanations: CB (commercial broiler chickens); BB (broiler breeders); CL (commercial layers); ND (no data)

age depending on the purpose of production (Tab. 1). Wilczyński et al. (19) isolated 4 enterococci (*E. cecorum*, *E. faecalis*, *E. hirae*, *E. faecium*) in older broilers (5 days up to 6 weeks old), and *E. cecorum* was the dominant species.

A total of 7 enterococcal species were retrieved from poultry production environmental samples (Fig. 3). *Enterococcus faecalis* (65.6%) and *E. faecium* (13.6%) were the most frequently isolated. Our results were consistent to Hayes et al. (6) who isolated *E. faecalis* (> 50%) and *E. faecium* (> 30%) from the poultry production environment, while 5 other species (*E. gal-*

linarum > *E. hirae* > *E. durans* > *E. casseliflavus*, *E. avium*) were less isolated. In opposition to above authors, we found *E. cecorum* (6%) in environmental samples.

In conclusion, this study indicates the high prevalence of bacteria of the *Enterococcus* genus in poultry. The present findings demonstrate the differences in *Enterococcus* species between poultry groups, including with regard to age. In total 10 enterococcal species (*E. faecalis*, *E. cecorum*, *E. hirae*, *E. faecium*, *E. gallinarum*, *E. casseliflavus*, *E. durans*, *E. avium*, *E. thailandicus*, *E. aquimarinus*) were detected in

Fig. 2. Percentage of *Enterococcus* species isolated from hatching eggs and dead-in-shell embryos Fig. 3. Percentage of *Enterococcus* species isolated from environmental samples

poultry, poultry environmental samples, hatching eggs and dead-in-shell embryos. *Enterococcus faecalis* and *E. cecorum* were found in all above-mentioned sources.

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