Full paper

Surveillance of the source of poultry infections with *Enterococcus hirae* and *Enterococcus cecorum* in Slovenia and *E. hirae* antibiotic resistance patterns

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Short title: *Enterococcus hirae* and *Enterococcus cecorum* in poultry

SUMMARY

Enterococcus cecorum and *Enterococcus hirae* can cause locomotor problems, septicaemia, and endocarditis in broiler chickens. Understanding transmission routes and resistance patterns are essential for effective treatment. The aim of this study was to follow the same animals from the breeder flock to the hatchery and up to 14-day-old broiler chickens on the farm to find the source of *E. cecorum* and *E. hirae*. During the production cycle, only faeces and organs of broilers were *E. hirae* positive in all three sampled farms in which recurrent enterococcal infections were previously confirmed. None of the isolates possessed virulence genes. Based on resistance profiles, a variety of different strains were present in faeces and organs of different broilers' ages. Samples from the breeder flock and hatchery were negative. Faecal shedding on the farm and tolerance of enterococci to the environmental conditions enable persistence of pathogenic enterococci in farm dust; therefore, adequate cleaning and disinfection after depopulation of the farms could prevent disease recurrence in the new cycle. Susceptibility testing of *E. hirae* isolates showed no resistance to the drugs of choice for the treatment of enterococcal infections in poultry.

Key words: Enterococcus hirae, *Enterococcus cecorum*, broiler chicken, antimicrobial resistance, route of infection, virulence genes

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INTRODUCTION

Poultry production is one of the most important food processing industries. In recent years, the poultry meat market has been growing throughout the world due to increasing consumer demand. Therefore, new adjustments and improvements in poultry production are needed. In intensive poultry production, bacterial infections are a growing problem, especially after the European Union's ban of the use of antimicrobial growth promoters (Cogliani et al., 2011). In the past few years, *Enterococcus cecorum* and *Enterococcus hirae* have been among the most common species of opportunistic pathogenic bacteria in poultry. Although enterococci could be a part of normal intestinal microbiota in birds, *E. cecorum* and *E. hirae* are frequently isolated from broiler chickens with spondylitis, arthritis, osteomyelitis, lameness, and endocarditis (Chadfield et al., 2005; De Herdt et al., 2008; Kolbjørnsen et al., 2011; Velkers et al., 2011; Robbins et al., 2012). Outbreaks on poultry farms lead to increased mortality rates and substantial economic losses, which significantly increase the cost of production (Chadfield et al., 2005; Robbins et al., 2012). The emergence of the disease on farms requires immediate treatment aided by early diagnosis. Enterococci are intrinsically resistant to many antimicrobial agents; thus, the choice of appropriate therapy is limited. Preventive measures are even more effective, but it is necessary to know the critical points in production – when, where, and how different bacterial species occur. The aim of this study was to find the source and transmission routes of *E. cecorum* and *E. hirae* in the hatchery and in the broiler flock as well as to obtain information on susceptibility to antimicrobial agents most frequently used in poultry production. To this aim, a study was designed to follow each production step of the same animals (from the breeder flock to the hatchery and up to 14-day-old broiler chickens on the farm).

MATERIALS AND METHODS

Sampling

Initially, faecal samples were taken from three parent Ross 308 broiler breeder flocks (followed through the production cycle in this study) and from two Lohmann layer breeder flocks, while eggs from all breeding flocks were hatched in the same hatchery. The sampled flocks consisted of 3800 to 4400 birds (Ross 308) and 6500 to 7500 birds (Lohmann), respectively. Faecal samples of floor-reared birds were collected from four barns for each broiler breeder flock (n =3; a total of 12 samples) and from one barn of each layer breeding flock (n=2). The age of broiler breeder flocks was 42, 53 and 28 weeks, while the layer breeder flocks were 38 and 61 weeks old. No antibiotics were used in the Lohmann layer breeder flocks or in the Ross 308 broiler breeder flock of 28-week-old birds. In the remaining two broiler breeder flocks, oxytetracycline was used four and seven months prior to

sampling, respectively. Feed was also sampled for breeding flocks and broilers used during the studied production cycle (n=10). Feed was initially sampled in feed silo and then on the farms.

Eggshells of each broiler breeder flock originating from individual barns (four barns per flock) were swabbed with sampling sponge (3M, USA) three times – in the barn before transport to the hatchery (n=12), in the hatchery before placing the eggs (n=12) into the incubator, and before placing eggs into the hatcher (n=12). At every stage, approx. 120 eggs from each of the four barns for every broiler breeder flock were swabbed. After hatching, eggshells (one sample per broiler breeder flock, n=3) and dead-in-shell (four to five per broiler breeder flock were pooled into one sample, n=3) samples were taken for microbiology tests.

In the hatchery, air sampling was performed on four locations where higher amounts of dust were present in the air: in the hatcher, at the location where chickens are inspected and sorted, in the storage room for chickens, and in the storage room for eggs. Air samples were taken on the days when eggs from a certain flock were hatched. In total, 12 air samples were taken. One flock was hatched just one day after hatching eggs for pullets. It was suspected that the hatchery could have a higher contamination level than usual. The Coriolis Air Sampler (Bertin Instruments, France) was used to sample air for 15 minutes at each location. Between 200 and 300 l of air per minute were filtered to 15 ml of saline solution and salt broth (Mueller-Hinton Broth supplemented with 6.5% sodium chloride; Oxoid, UK).

Broilers were housed on three farms (A, B, C), where recurrent enterococcal infections had previously been confirmed, but were not diagnosed during the studied production cycle. The chickens were not treated with antibiotics during the study. Before animal housing, the environment of all three farms was sampled. On every farm, ten swabs in empty barns after cleaning and disinfection (walls and floor), five swabs of feeders, and ten swabs of drinking nipples were collected. The same types of swabs from the same farm were pooled in the laboratory. Additionally, litter (n=3) and water (n=6) were sampled on each farm. When the broilers were housed, faeces mixed with litter and organs (heart, liver) of up to five dead animals were taken every two to three days until the broilers were 14 days old. Faeces and organs were processed in the laboratory within three hours after sampling. A total of 12 samples per farm was collected.

Based on the results of farms A–C, where enterococcal infections were not observed, additionally, with the aim of finding an environmental persistence of *E. cecorum/E. hirae*, a total of 15 samples was collected from four different broiler farms (D–G) where clinical signs characteristic of *E. cecorum/E. hirae* infection were observed. On farms D, E, and F samples were taken after the flocks were removed from the barns (farm D – faeces mixed with litter; farm E and F – swabs of the

environment and equipment), while sampling from farm G was possible only during the production cycle. Sampling of dust, feed, chicks' hearts, liver, and feet was performed.

All types of samples are presented in Table 1; a total of 147 samples was subjected to bacteriological examination.

Bacteriology

Faeces, litter, feed, and eggshells were mixed with saline solution (ratio 1:1) in a 500 ml plastic container, shaken for one hour and then left to settle. Supernatant was used for bacteriological analysis. Sampling sponges and swabs were washed in 50 ml or 500 µl of saline solution, respectively, and processed as described below. Organs of daily dead and dead-in-shell chicks were homogenized using a stomacher. For all sample types, one loop (10 µl) of the sample was directly plated onto 5% sheep blood agar (Columbia Blood Agar Base; Oxoid, UK), chromogenic Uri*Select* agar plates (Bio-Rad, France), and 1 ml of supernatant was inoculated into 9 ml of salt broth. One litre of water was filtered through a 0.22 µl membrane filter (Corning, USA) which was swabbed, and the swab contents inoculated onto 5% sheep blood agar and Uri*Select* agar plates. Broth was incubated at 37ºC for 24h, and then 10 µl of the broth was inoculated onto Uri*Select* agar plates. Plates were incubated under microaerophilic and aerobic conditions at 37ºC and examined after 24 h and 48 h.

Based on colony morphology and colour on the Uri*Select* agar plates, suspect colonies (approx. 3 to 5) were subjected to identification. Biochemical tests for esculin, mannitol, raffinose, arginine dihydrolase (ADH), and sucrose were carried out to differentiate *E. cecorum* and *E. hirae* from other *Enterococcus* species. Bacterial species were confirmed using multiplex PCR targeting *E. hirae* and *E. cecorum sodA* genes (Jackson et al., 2004). The 16S rDNA gene was sequenced for all suspect colonies with a PCR-negative result (Lane et al., 1985). The amplicon quality was checked using the QIAxcel capillary electrophoresis system (Qiagen, Germany) before sequencing at SEQMe (Czech Republic). DNA sequence analysis was performed using the BLAST tool of the National Center for Biotechnology Information (NCBI). All colonies (one to four colonies per sample) confirmed as *E. hirae* or *E. cecorum* were subjected to further analysis.

Detection of virulence factors using multiplex PCR

All *E. hirae* (n=49) and *E. cecorum* (n=3) isolates were tested for enterococcal virulence factors – adhesion of collagen protein (*ace*), endocarditis antigen (*efaA*), aggregation substance (*asa1*), gelatinase (*gelE*), cytolysin (*cylA*), enterococcal surface protein (*esp*), and hyaluronidase (*hyl*) (Vankerckhoven et al., 2004; Martin-Platero et al., 2009). Suspensions of the bacterial culture were incubated for 15 min at 95ºC and supernatants obtained after centrifugation (2 min at 12000 rpm) were used as sources of DNA for PCR. Virulence genes were detected using two multiplex PCR tests

and the Multiplex PCR Kit (Qiagen, Germany) according to the previously published protocol and manufacturer's instructions (Martin-Platero et al., 2009).

Antimicrobial susceptibility testing (AST)

All *E. hirae* isolates (n=49) were tested for susceptibility to the 19 antimicrobial agents most frequently used in poultry (Table 2). The broth microdilution method was performed on commercially available Avian Plates AVIAN1F (Trek Diagnostic Systems, UK) according to the manufacturer's instructions. Additionally, the susceptibility of 49 isolates to amoxicillin with clavulanic acid was tested with the E-test (bioMerieux, France) according to the manufacturer's instructions. Breakpoints were defined according to the Clinical and Laboratory Standards Institute CLSI recommendations and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (CLSI, 2014; EUCAST, 2017). The reference strain of *Enterococcus faecalis* ATCC 29212 was used for quality control of both procedures.

RESULTS

Faecal samples from layer and parent broiler breeder flocks were negative for *E. hirae* and *E. cecorum*. Among all samples taken during the entire production process on farms A–C (feed, eggshells, dead-in-shell, air samples, environmental swabs, litter, water, faeces, organs), only faeces and organs of dead animals were positive for *E. hirae* on all three farms (A, B, C) (n=41). On farm A, organs were positive until day 5 (n=5), while faeces were positive until day 10 (n=13). On farm C, only faeces were positive until day 7 (n=3). On farm B, organs were positive until day 14 (n=13), but were negative on day 2, while faeces were positive on days 2, 5, and 12 (n=7) (Table 1).

Additionally, from all four farms (D–G) with clinical signs of disease, *E. hirae* was isolated from different samples (n=8). On farm G, where there was ongoing disease, *E. cecorum* was isolated from the organs (heart and liver) and feet (n=3), while *E. hirae* was found in heart (n=1) and dust samples (n=2). Feed was negative. From the other farms (D, E, F) with clinical signs of disease, samples were taken after the flocks were removed from the barns. *E. hirae* was isolated from all three farms from different swabs taken in barns (feeders, drinking nipples, floor; farms E and F; n=3) and from litter mixed with faeces (farm D; n=2). Furthermore, *E. hirae*-positive swab from farm E was taken after cleaning and disinfection of a barn, which was prepared for a new flock (Table 1).

The results of AST of the 49 *E. hirae* isolates tested (including MIC_{50} and MIC_{90}) are summarized in Table 2. A bimodal distribution was observed for erythromycin (ERY), oxytetracycline (OXY), tetracycline (TET), tylosin tartrate (TYLT), and trimethoprim-sulfamethoxazole (SXT).

Eight resistotypes were found on sampled farms (Table 3). Resistotypes were determined according to the MIC results for seven antimicrobials – ERY, OXY, TET, TYLT, enrofloxacin (ENRO), penicillin (PEN), and SXT, which differed among the isolates. All isolates were resistant to sulphadimethoxine and sulphathiazole. Antimicrobials connected with intrinsic resistance in enterococci were not considered in resistotype determination. All isolates were resistant to at least one of the seven antimicrobials mentioned above, except *E. hirae* isolates from the farms with clinical problems (farms E and G), which were susceptible to the seven antimicrobial agents mentioned above. *E. hirae* isolated from environmental dust and the heart of the diseased chick, on farm G, showed the same resistance pattern. Distribution of resistotypes on different farms at different chick ages is presented in Table 3. Up to four different resistotypes were detected in the same faecal samples (one to four colonies per sample underwent AST). The occurrence of the same resistotype in faeces and organs at the same age was observed twice in five-day-old chicks on farms A and B. Two resistotypes were found on all three farms (ERY-TYLT-ENRO and TET-ENRO-PEN) but isolates from farms A and B were resistant to more antibiotics than were the isolates from other farms.

Virulence genes *efaA*, *ace*, *asa1*, *gelE*, *cylA*, *esp*, and *hyl* were not detected in any of the *E. hirae*/*E. cecorum* isolates tested.

DISCUSSION

Enterococcal infections in broilers have a considerable economic impact on poultry production. Young animals are particularly susceptible to the disease, which is frequently seen as lameness in the flock. Before this study, enterococcal spondylitis and bacteraemia were observed on farms A–C in one- to two-week-old broiler chickens. According to the resistance patterns, a variety of different strains were present in faeces and organs from farms A–C, which suggested that different types of pathogenic enterococci are part of the chickens' normal microbiota, and a combination of different factors (e.g., emergence of strains with increased pathogenicity, environmental conditions, hosts' predisposing factors, and underlying diseases) could provoke the disease (Velkers et al., 2011). However, conclusions regarding the pathogenicity of the *E. hirae* strains isolated from farms without clinical signs could not be drawn.

The infection routes and the modes of transmission are not clear. Vertical transmission from the breeder stock seems unlikely (Robbins et al., 2012). Previous studies have suggested different routes and pathogenesis of the infection. One of the suggested risk factors is the temperature of egg incubation impacting the development of the chicken's skeleton, which could be a predisposing factor for enterococcal infections (Oviedo-Rondon et al., 2009). *E. cecorum* and *E. hirae* are normally present in the gut and could enter the bloodstream via intestinal lesions, caused by previous enteric disease, and cause the infection (Borst et al., 2017). *E. cecorum* colonization of broilers during the

first week of life indicated future development of the disease, while broilers without the disease became colonized after three weeks of life (Borst et al., 2017). The bacteria could also enter the animal through the air sacs, as faecal shedding of enterococci occurs on the farm. Since enterococci are tolerant to a wide range of environmental conditions (e.g., extreme temperature, drying), persistence in farm dust was observed (Jung and Rautenschlein, 2014). In the present study, *E. hirae* was detected in dust and environmental samples of the emptied barns immediately after the flock with clinical signs of infection was removed. One barn was *E. hirae*-positive even after complete clean-out and disinfection. The reason could be the presence of *E. hirae* in biofilms in the farm environment, since *E. hirae* is a moderate biofilm producer capable of forming biofilms on hospital material surfaces (Di Lodovico et al., 2017). However, environmental samples from barns A–C, with confirmed repetitive enterococcal infections in previous flocks, were *E. cecorum*/*E. hirae* negative, which correlates with the absence of clinical signs characteristic of *E. cecorum*/*E. hirae* infection in studied flocks. The results suggest that repeated enterococcal outbreaks could be linked to insufficient cleaning/disinfection of the barns after the flocks were removed, as insufficient treatments in the barns could result in the recurrence of the disease in the new production cycle. Other routes of infection were also suggested in previously published studies. The source of *E. cecorum* was investigated in the farm environment (water lines, mice trapped on the farm, feed, litter…), including air in hatcheries, dead-in-shell, and hatching eggs, but all samples were *E. cecorum*-negative (Kense and Landman, 2011; Robbins et al., 2012). Jung et al. (2017b) reported enterococcal spondylitis in a newly built broiler house and detected *E. cecorum* in newly-hatched chicks. This observation suggested that transmission from the hatchery was more likely than an on-farm reservoir. However, in the present study, *E. cecorum*/*E. hirae* were detected neither in the hatchery nor in breeder flocks. Only *E. hirae* was isolated from faeces and broilers' organs, which could be explained by the selection of laboratory methods. Classical bacteriological cultivation and biochemical identification of a limited number of suspect isolates were performed. Although *E. cecorum* is part of the *Enterococcus* genus, its cultivation requirements are more demanding than those for other enterococci (Suyemoto et al., 2016). Thus, other fast-growing bacteria could overgrow *E. cecorum*. For this purpose, a real-time PCR screening method (Jung et al., 2017b), and/or cheaper, more reliable identification of the isolates with MALDI-TOF, could result in more *E. cecorum*-positive samples. Some studies have reported that intestinal colonization with *E. cecorum* begins at the age of 2–5 weeks. This finding may be the reason that *E. cecorum* isolation from faeces and organs of broilers failed in our study (Devriese et al., 1991; Stepien-Pysniak et al., 2016).

The absence of enterococcal virulence factors is in concordance with previously published studies, which reported low occurrences (up to 20%) of *esp*, *hyl*, *asa1*, *gelE*, *efaA*, and *ace* genes in *E. cecorum*

(Dolka et al., 2017; Jung et al., 2017a). To the best of our knowledge, the presence of virulence factors in *E. hirae* isolates from broiler chickens has not yet been reported.

AST is of utmost importance for successful antibiotic therapy at the onset of disease. Data on resistance profiles for *E. hirae* are scarce and are usually a part of more extensive studies dealing with *Enterococcus* sp. in different animal species (Diarra et al., 2010; Kojima et al., 2010; Stepien-Pysniak et al., 2016; de Jong et al., 2018). The drug of choice for the treatment of enterococcal infections in poultry is amoxicillin. Isolates resistant to this agent were not observed in the present study. Other European studies have also reported low resistance to amoxicillin (2.4–8.1%) (Stepien-Pysniak et al., 2016; de Jong et al., 2018). High resistance to ENRO (89.8%) was observed, which is in accordance with the Polish study (85.7%) (Stepien-Pysniak et al., 2016). De Jong et al. (2018) reported two avian strains with high-level resistance to gentamicin (which was not tested in the present study), but otherwise MIC_{50} (=4) and MIC_{90} (=8) were lower than in the present study (>8). Bimodal distribution for ERY, TET, OXY, and TYLT has also been reported in previous studies (Stepien-Pysniak et al., 2016; de Jong et al., 2018), although the percentage of strains resistant to these antibiotics varied. Lower resistance to TET (28.6% vs. 38–65%) and TYLT (44.9% vs. 61.9%) was observed in the present study, while resistance to ERY (53.1 %) was higher than in the data reported by nine EU countries (37.8%) (Stepien-Pysniak et al., 2016; de Jong et al., 2018). In isolates from Canada, resistance to TET (88.6%), streptomycin (33.3%), and PEN (55.6%) was observed at a significantly higher frequency (Diarra et al., 2010), while resistance to ERY (55.6%) and TYLT (55.6%) was comparable to results described herein. As resistance to the different antimicrobial groups in *E. hirae* was observed, it should not be disregarded that *E. hirae* could be the reservoir for antibiotic resistance genes, which are encoded in a variety of mobile genetic elements (Ahmed and Baptiste, 2018). These mobile genetic elements could be transferred from commensal strains to pathogenic bacteria or resistant *E. hirae* from broilers could enter the food chain. Contaminated meat could play an important role in human disease, as *E. hirae* have become a clinically significant pathogen (Brayer et al., 2019). A variety of different *E. hirae* strains were detected in the faeces and organs of broilers and could also persist in farm dust. Adequate biosecurity measurements on the farm and controlled conditions in the hatchery and on the farm are necessary for the prevention of enterococcal disease in broilers. Data on antimicrobial resistance patterns is critical to prevent empiric treatment of animals in the case of disease outbreak to reduce the costs of poultry production and to minimize the risk of antimicrobial resistance development. In an era of increasing antimicrobial resistance, low resistance of *E. hirae* to the drugs of choice for enterococcal infections in poultry is encouraging.

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Table 1. Results of the presence of *Enterococcus hirae* in different samples and locations

				Farms with previously confirmed recurrent enterococcal infections			Farms with observed clinical signs characteristic of enterococcal infections
	Layer breeder flocks (n=2)	Broiler breeder flocks (n=3)[a]	Hatchery	Broiler farm A	Broiler farm B	Broiler farm C	Broiler farms D–G
Feed	neg	neg	-	neg	neg	neg	farm G – neg
Eggshells before hatching[b]	-	-	neg	-	-	-	-
Eggshells after hatching (one pool per flock)	-	-	neg	-	-	-	-
Dead-in-shell (one pool per flock)	-	-	neg	-	-	-	-
Air (four locations, three times)	-	-	neg	-	-	-	-
Farm environment before animal housing[c]	-	-	-	neg	neg	neg	-
Farm environment after or during production cycle[d]	-	-	-	-	-	-	farm E – pos farm F – pos farm G – pos
Faeces mixed with litter	neg	neg	-	day 3 – pos day 5 – pos day 7 – pos day 10 – pos day 12 – neg day 14 – neg	day 2 – pos day 5 – pos day 7 – neg day 9 – neg day 12 – pos day 14 – neg	day 2 – pos day 4 – pos day 7 – pos day 9 – neg day 11 – neg day 14 – neg	farm D – pos

Organs (heart, liver)	-	-	-	day 3 – pos day 5 – pos day 7 – neg day 10 – neg day 12 – neg day 14 – neg	day 2 – neg day 5 – pos day 7 – pos day 9 – pos day 12 – pos day 14 – pos	day 2 – neg day 4 – neg day 7 – neg day 9 – neg day 11 – neg day 14 – neg	farm G – pos[e]

neg: negative for *E. hirae*; pos: positive for *E. hirae*

[a]Four barns for each breeder flock.

[b]Eggshells before hatching were sampled for each broiler breeder flock and barn in three locations on the farm and in the hatchery.

[c]Wall and floor swabs of empty barns after cleaning and disinfection, swabs of feeders and drinking nipples, litter and water.

[d]Swabs of the environment (dust) and equipment.

[e]*E. hirae* and *E. cecorum* were isolated from the organs.

Table 2. Distribution of MICs and antimicrobial susceptibility of *Enterococcus hirae* isolates

antimicrobials	number of strains with MIC (µg/ml) (n=49)[a]																	range	MIC_{50}	MIC_{90}	resistance (%)
	≤0.06	***0.06***	***0.12***	***0.25***	***0.5***	***1***	***2***	***4***	***8***	***16***	***32***	***64***	***128***	***256***	***512***	***1024***	***>1024***				
enrofloxacin					4		1	44										0.5->2	>2	>2	44 (89.8)
gentamicin								1	12	36								4->8	>8	>8	[b]
ceftiofur									49									>4	>4	>4	[b]
neomycin									2	5	32	10						8->32	32	>32	[b]
erythromycin		23							26									≤0.12->4	≤0.12	>4	26 (53.1)
oxytetracycline						35				14								1->8	1	>8	14 (28.6)
tetracycline					35					14								0.5->8	0.5	>8	14 (28.6)
amoxicillin			10		35			3	1									≤0.25-8	0.5	0.5	0
spectinomycin												49						64	64	64	0
sulphadimethoxine															49			>256	>256	>256	49 (100)
trimethoprim/ sulfamethoxazole				39				10										≤0.5/9.5->2/38	≤0.5/9.5	>2/38	10 (20.4)[c]
florfenicol							31	18										2-4	2	4	0
sulphathiazole															49			>256	>256	>256	49 (100)
penicillin					3	23	19			4								0.5->8	1	2	4 (8.2)
streptomycin											1	15	33					16-64	64	64	0
clindamycin								2	47									4->4	>4	>4	[b]
novobiocin				49														≤0.5	≤0.5	≤0.5	0
tylosin tartrate (µg/ml)							***2.5***	***5***	***10***	***20***											
						27					22							≤2.5->20	≤2.5	>20	22 (44.9)
amoxicillin/ clavulanic acid (µg/ml)	***≤0.016***	***0.016***	…	***0.094***	***0.125***	***0.19***	***0.25***	***0.38***	***0.5***	***0.75***	***1***	***1.5***	***2***	***3***	***4***	***6***	***8***	…				
					7	22	16	1					3						0.125-2	0.19	0.25	0

[a]The shaded areas represent the range of antimicrobial concentrations tested. Vertical lines denote CLSI or EUCAST (for amoxicillin, amoxicillin/clavulanic acid, and trimethoprim/sulfamethoxazole) recommended breakpoints.

[b]Enterococci are intrinsically resistant to low concentrations of aminoglycosides, clindamycin, and cephalosporins. High concentrations of aminoglycosides were tested only for streptomycin.

[c]Trimethoprim-sulfamethoxazole is not clinically effective against enterococci, although *in vitro* result may show susceptibility.

Table 3. Distribution of *Enterococcus hirae* resistotypes on different farms in relation to the isolates' origin and chickens' age

	Farm A		Farm B		Farm C		Farms D, E, F, G[c]	
	Feces	Organs	Feces	Organs	Feces	Organs	Environment	Organs
Resistotype[a]	**Age of chicks in days** (number of tested isolates[b])		**Age of chicks in days** (number of tested isolates[b])		**Age of chicks in days**		**Farms** (number of tested isolates[b])	
ERY-SXT-TYLT-ENRO	**3** (2)/**5** (2)/**7**	**5** (2)		**5** (2)				
ERY-TET-TYLT-ENRO	**3/5/7**			**7**				
ERY-TYLT-ENRO	**7/10** (2)		**5** (2)/**12**	**14**	**2**			
ERY-TYLT-PEN							**D**	
TET-SXT-ENRO				**5**				
TET-ENRO-PEN	**3**			**14**	**7**			
TET-ENRO	**7**	**3** (3)	**2**				**D**	
ENRO			**2** (2)/**5**	**5/9** (2)/**12** (4)	**4**		**F** (2)	
Susceptible							**E/G** (2)	**G**
Total number of tested isolates (n=49)	13	5	7	13	3	0	7	1

ENRO: enrofloxacin; ERY: erythromycin; PEN: penicillin; SXT: trimethoprim/sulfamethoxazole; TET: tetracycline; TYLT: tylosin tartrate

[a]Excluding antibiotics connected with intrinsically resistance in enterococci and sulphadimethoxine, sulphathiazole as all isolates were resistant to these two antibiotics.

[b]Indicated only if more than one isolate was tested.

[c]Farms D, E, F, G with observed clinical signs characteristic of *E. cecorum*/*E. hirae* infection