Enterococci with Acquired Vancomycin Resistance in Pigs and Chickens of Different Age Groups

PATRICK BUTAYE,1* LUC A. DEVRIESE,1 HERMAN GOOSENS,2 MARGARETHA IEVEN,2 and FREDDY HAESEBROUCK1

Faculty of Veterinary Medicine, University of Ghent, B-9820 Merelbeke,1 and Department of Clinical Microbiology, Antwerp University Hospital, U.I.A., B-2650 Edegem,2 Belgium

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A correlation between the use of the glycopeptide antibiotic avoparcin as a growth promoter in farm animals and the occurrence of vancomycin-resistant enterococci (VRE) in the intestines in Germany (5) and in Denmark (1) has been described. In Belgium and other countries of the European Community, the use of avoparcin was suspended in April 1997 for a period of 2 years.

Surveys on VRE prevalence in animals published in recent years have concentrated on the possible influence of avoparcin feeding, disregarding other factors which may be of importance. Since the intestinal flora of animals changes with age, we wanted to investigate the occurrence of VRE in pigs and poultry of different types and in different age groups.

A total of 180 ex recto fecal samples were collected from 60 healthy sows, 60 piglets, and 60 fattening pigs on six farms in Belgium 5 to 11 months after the ban on avoparcin use became effective. Piglets (at all of six farms) and fattening pigs (at five of six farms) were fed other nutritional antibiotics, but the sows were not. No reliable data for previous avoparcin use could be obtained except the information that this antibiotic never had been used in the sows.

Four farms with laying hens were sampled in 1997 and 1998. A total of 120 samples were derived from three age groups on each farm: chicks from 2 to 5 weeks old, chicks from 10 to 15 weeks old, and chickens in production (over 22 weeks old). Only one layer farm used a growth promoter (the streptogramin antibiotic virginiamycin) for animals not in production. The other farms had never included any growth-promoting antibacterial in the feed.

Animals at four broiler chicken farms that used various growth-promoting antibiotics were sampled for the presence of VRE in 1997. On each farm 80 samples were collected from four groups of 10 broilers less than 3 weeks old (7 to 12 days old) and four groups of 10 broilers more than 3 weeks old (28 to 35 days old). One broiler farm was known to have used avoparcin feeding until 8 months before sampling took place.

Samples were inoculated within 2 h after collection. They were suspended at 1/10 (wt/vol) in phosphate-buffered saline. By the direct plating technique, 1-mg (1-mg) calibrated loops were used to inoculate Slanetz and Bartley (Oxoid, Basing-stoke, United Kingdom) agar plates supplemented with 6 μg of vancomycin/ml, allowing semiquantitative analysis. By the enrichment method, 1 ml (approximately 0.1 g) of each suspension was inoculated into 10 ml of kanamycin aesculin azide (LabM, Bury, United Kingdom) broth supplemented with 6 μg of vancomycin/ml and incubated at 37°C for 2 days. The samples found positive for VRE by direct plating contained at least 10,000 CFU of VRE/g (1 μl of a 1/10 suspension was inoculated), whereas samples positive only after enrichment contained between 10 and 10,000 CFU of VRE/g and negative samples contained fewer than 10 CFU of VRE/g.

Phenotypic confirmation of vancomycin resistance and species identification were carried out as described by Devriese et al. (3). Vancomycin resistance genes (vanA, vanB, vanC1, and vanC2) were determined for 21 pig strains and 34 chicken strains by using a PCR method (3, 4). Since Enterococcus casseliflavus and E. gallinarum exhibit natural low-level glycopeptide resistance due to the presence of the vanC gene, only strains showing high-level resistance due to the acquisition of the vanA gene were included.

Statistical analysis was performed by the chi-square test for comparing prevalences and for comparing the numbers of broilers with different amounts of VRE per gram of feces by using the statistical program Statistix (Analytical Software). A significance level of P ≤ 0.05 was used.

In pigs, no VRE were isolated by direct culture on vancomycin plates but enrichments yielded 31 vancomycin-resistant E. faecium strains and 1 E. hirae or E. durans strain from sows (53% of sows were positive), 13 vancomycin-resistant E. faecium strains from piglets (21% of piglets were positive), and 11 vancomycin-resistant E. faecium strains from fattening pigs (18% of fattening pigs were positive). All 21 pig VRE strains examined with the PCR method were found to carry the vanA gene. There were significant differences in prevalence of VRE between sows and piglets (P ≤ 0.05) and between sows and fattening pigs (P ≤ 0.05).

Most surprisingly, the pigs in which the use of growth promoters is prohibited (the sows) showed the highest prevalence of VRE. The reason for this remains unclear. The possibility cannot be excluded that these animals had received avoparcin when young.

Thirty-nine of 40 broilers less than 3 weeks of age and 37 of 40 broiler chicks more than 3 weeks of age were positive by the direct method as well as by the enrichment method. A total of 116 VRE strains were isolated from the 80 samples of broiler feces. Fifty-six (48%) of the VRE strains were E. faecium, 49 (42%) were E. hirae or E. durans, and the remaining strains were E. faecalis (4 strains), E. casseliflavus (2 strains), and E. gallinarum (5 strains). Up to four different VRE species were...
found in the same flock and, frequently, different VRE species were present in a single animal. The results of semiquantitative analysis are shown in Table 1. The younger animals carried significantly more VRE than the older animals ($P < 0.05$). All laying hens more than 10 weeks of age were negative for VRE by both isolation procedures. Of laying chicks between 2 and 5 weeks of age, five (12.5%) were positive for VRE. All these strains were $E. hirae$ or $E. durans$. PCR analysis revealed the presence of the vanA gene in all of the 34 isolates from chicks examined.

In chicks younger than 1 week old, $E. faecalis$ and $E. faecium$ are the predominant enterococcal components of the intestinal flora. After 1 week of age the flora shifts towards $E. faecium$, $E. hirae$, and $E. durans$, and later on $E. cecorum$ largely predominates (2). The latter species is capnophilic and does not grow on enterococcal selective media. This shift may explain in part the failure to find VRE in laying chickens over 10 weeks of age. However, the prevalence of VRE and the number of VRE per animal in broilers were much greater than in laying type chicks of the same age group. Avoparcin has been used in the past in broilers but not in laying chicks and hens, for which only the growth promoters bacitracin, virginiamycin, and bambermycin are allowed. This might indicate a selective effect of a previous use of avoparcin in these animals. However, as the use of avoparcin was already prohibited at the time of sampling in poultry, the very high levels of VRE indicate that this type of resistance is disappearing only slowly after the removal of the antibiotic.

Comparison of results reported in the literature is hampered by the differences in methods used. The findings reported here demonstrate that in low-prevalence populations, such as pigs and laying chickens, isolation rates revealed by enrichments strongly differ from those obtained by direct isolation on selective vancomycin-supplemented plates.

A second conclusion to be drawn from the present results is that in reports and discussions on the occurrence of VRE in chickens and pigs, precise data on the age and type of animals are of utmost importance. Comparisons between treatment groups and between geographic regions cannot be made unless age groups and isolation methods are similar.

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