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Intestinal carriage of amoxicillin and vancomycin-resistant Enterococcus faecium in humans an pets from the Dutch community

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Background: Amoxicillin-and vancomycin-resistant *E. faecium* (ARE and VRE) are increasingly observed in hospitalized patients in the Netherlands and are thought to be hospital-acquired. As the prevalence of ARE and VRE carriage in humans and pets in the community is unknown, we determined prevalence, risk-factors and co-carriage of ARE and VRE.

Material/methods: From November 2014 to November 2015, a random stratified (by region, population density and degree of urbanization) sample of ~2000 inhabitants of the Netherlands was drawn monthly from Dutch population registry. Subjects were invited to complete an online questionnaire and to provide a faecal sample. If applicable, subjects were asked to submit one sample from their dog or cat. Faecal samples were enriched with Enterococcosel broth (12 mg/L aztreonam and 16 mg/l amoxicillin or 4 mg/L vancomycin) and plated on Enterococcosel plates with amoxicillin (16 mg/L) or vancomycin (4 mg/L). Colonies were plated on blood agar and MALDI-TOF speciation was performed. Presence of *vanA* and *vanB* genes was determined by PCR. Core Genome Multilocus Sequence Typing (cgMLST) was performed to analyze genetic relatedness between human and pet isolates by comparing cluster types (CT) (defined as ≤20 alleles difference per CT). Logistic regression

analyses with internal validation by bootstrapping were performed to determine risk factors for ARE carriage.

Results: In total 25,365 subjects were invited to participate, of which 4,721 (18.6%) completed the questionnaire. 1,992 (42.2%) human, 277 dog and 118 cat faecal samples were submitted, and ARE were detected in samples from 29 humans (1.5%, 95%CI:1.0-2.1), 71 dogs (25.6%, 95%CI:20.8%-31.1%) and 6 cats (5.1%, 95%CI:2.4%-10.7%). There was no evidence of regional clustering of ARE carriage (Figure 1). VRE (*vanA*) were detected in 1 human and 2 dog samples. ARE or VRE were not detected in the 388 paired samples from humans and pets. Antibiotic usage within 8 weeks prior to sample collection was a risk factor for ARE carriage in humans (OR:4.0, 95%CI:1.4-10.6). In dogs risk factors were eating raw meat (OR:3.8 95%CI:1.7-8.3) and the use of antibiotics in the past six months (OR:2.4, 95%CI:1.2-5.0). cgMLST analysis identified 68 different CTs, including 27 among human and 45 among pet isolates; 4 CTs were shared among human (n=7, 23.3%) and pet (n=21, 26.3%) isolates.

Conclusions: In the Netherlands the prevalence of intestinal carriage with ARE and VRE in non-hospitalized humans is 1.5% and 0.1%, respectively. The prevalence of ARE carriage was 25.6% in dogs and 5.1% in cats. Prior antibiotic use was associated with ARE carriage in humans and dogs, as was eating of raw meat for dogs. Co-carriage of ARE and VRE was not observed. cgMLST analysis yielded a separation between human and pet CTs, suggesting limited transmission between human and pets.

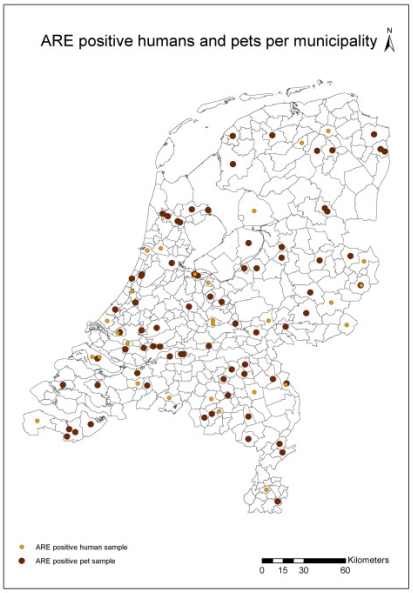

Figure 1. ARE distribution in the community