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Intestinal carriage of ampicillin- and vancomycin-resistant *Enterococcus faecium* in humans and pets in the Dutch general population

National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

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ECCMID - 2017

Transparency Declaration

- None

Vancomycin-resistant & Ampicillin-resistant Enterococcus faecium

VRE: Vancomycin-resistant *Enterococcus faecium*
ARE: Ampicillin-resistant *Enterococcus faecium*

- *Enterococcus faecium* exposure to antibiotics:
  - changes in the gut microbiota
  - facilitate colonization of the GI tract by VRE/ARE (Bonten M.J.M., 2001)
- First outbreak in Europe in 1988 (Leclercq R., et al. 1988)

What is known about the prevalence

Hospitals

- 2012-2015: 44 VRE outbreaks in Dutch hospitals
- 2015: incidence of VRE in Dutch hospitals ~1% (Nethmap, 2016)

Community

- 1996: study to VRE in turkey farmers and area residents
  - 14% area residents (van den Boogaard, 1997)
- 2002: VRE not observed in dogs in the Netherlands (Wagenvoort J., et al., 2002)
- 2006-2007: ARE in healthy dogs
  - 23% in UK
  - 76% in Denmark (Damborg P. , et al., 2009)

→ **What is the current status of VRE/ARE in the community?**

Aims

Determine:

1. the prevalence of ARE and VRE in humans, dogs and cats
2. risk factors for ARE and VRE carriage in humans, dogs and cats
3. the co-carriage of ARE and VRE between human-pet pairs
4. The genetic relatedness of non-hospitalized humans and pets

Study design

Cross-sectional

- ESBLAT populationstudy
- from October 2014 to October 2015
- Monthly-repeated
- Online questionnaires
- Random faecal samples of ~2000 Dutch inhabitants
  - and their dog or cat

Laboratory methods

- Plated on Enterococcus plates:
  - Amoxicillin (16 mg/L)
  - Vancomycin (4 mg/L)
- Susceptibility testing with Roscotablet diffusion method.
- PCR: *vanA* and *vanB* (vancomycin)

Invitation & response

Humans

25,365 Invited

4,721 (18.6%) completed questionnaire

1992 (42.2%) Faecal samples submitted

Pets

395 (277 dogs **& 118** cats**)**

Prevalence of ARE/VRE in humans, dogs and cats

Humans

- **VRE**:
  - 1 case
  - *VanA* gene.
  - prevalence: 1/1992=**0.05%**
- **ARE**: 29 cases
  - Prevalence:

29/1992= **1.5%** (95% CI: 1.0-2.1)

Dogs

- **VRE**:
  - 2 dogs
  - *VanA* genes.
  - Prevalence: 2/277=**0.7%**
- **ARE**: 66 dogs
  - Prevalence dogs:

71/277=**25.6%** (95% CI: 20.8-31.1%)

Cats

  - Prevalence cats:

6/126= **4.8%** (95% CI: 2.2%-10.0%)

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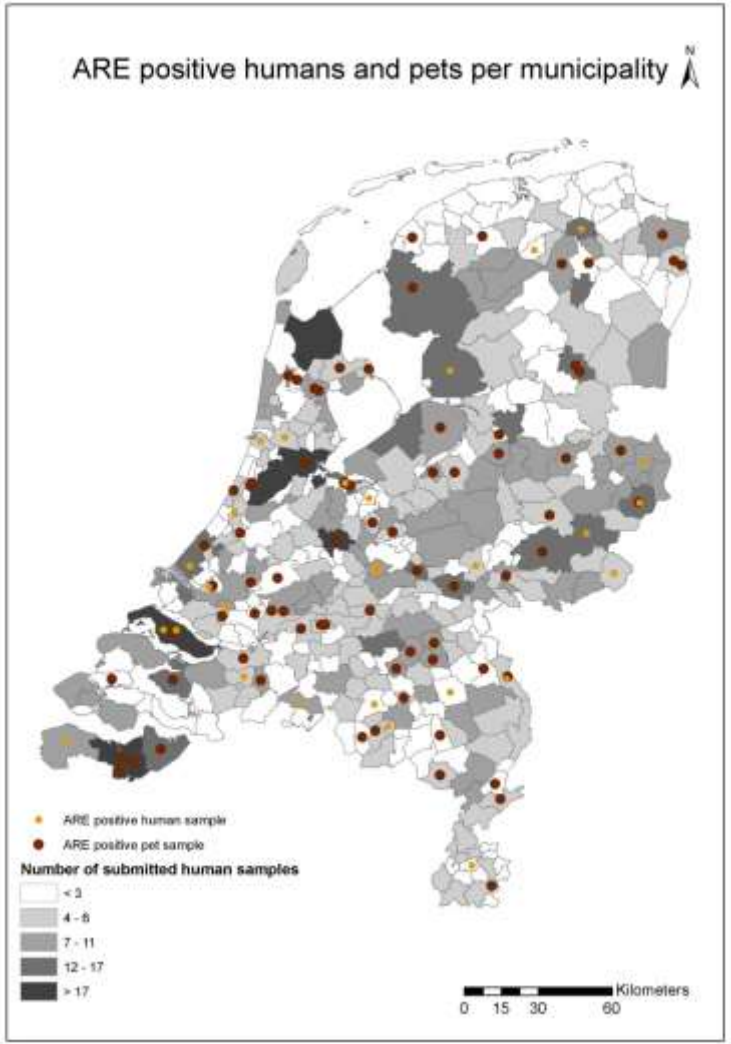
Geographical distribution of ARE



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Annemieke Mulder

Risk factors for ARE and VRE carriage in humans, dogs and cats

- Multivariable logistic regression models:
  - for humans
  - for dogs

Potential risk factors ARE for human

- Age
- Level of education
- Gender
- Country of birth is the Netherlands
- Use of antibiotics
- Use of proton pump inhibitors
- Use of other drugs
- Hospitalization
- GI complaints
- Travel
- Animals in/around home
- Dogs/cats in households
- Direct contact
- Ate raw or undercooked meat

Risk factors for ARE in humans

Variables	Univariate OR (95% CI)	Multivariate OR (95% CI)	Bootstrapped multivariate OR (95% CI)
age			
<18	Reference	reference	reference
≥18	0.6 (0.2-1.4)	0.4 (0.2-1.1)	0.5 (0.2-1.3)
use of AB in past 8 wk	**4.7 (1.9-11.2)**	**4.6 (1.9-11.5)**	**4.2 (1.7-11.2)**
use of proton pump inhibitors in past 6	**2.7 (1.2-5.8)**	**2.7 (1.2-6.2)**	**2.7 (1.1-6.3)**
travel abroad in past 12 m	0.6 (0.3-1.2)	0.4 (0.2-1.1)	

Potential risk factors ARE for dogs

- Age
- Gender
- Hospital
- AB use
- GI symptoms
- Travel
- Contact other animals
- Catch prey
- Coprophagy (eating of stool)
- Eat raw meat
- Eat wet food
- Eat dry food

Risk factors for ARE in dogs

Variables	Univariate OR (95% CI)	Multivariate OR (95% CI)	Bootstrapped multivariate OR (95% CI)
Use of AB in past 6m	**2.2 (1.1-4.4)**	**2.3 (1.1-4.8)**	**2.3 (1.1-4.6)**
Dog eat raw meat	**3.2 (1.6-6.7)**	**3.2 (1.5-6.7)**	**3.2 (1.4-6.6)**
Dog eat stool	1.5 (0.2-2.7)	1.5 (0.8-2.9)	1.5 (0.8-3.0)

Co-carriage of ARE in human-pet pairs

- Is there a relation between humans and pets regarding ARE prevalence?
  - **384** human-pet pairs belonging to the same household

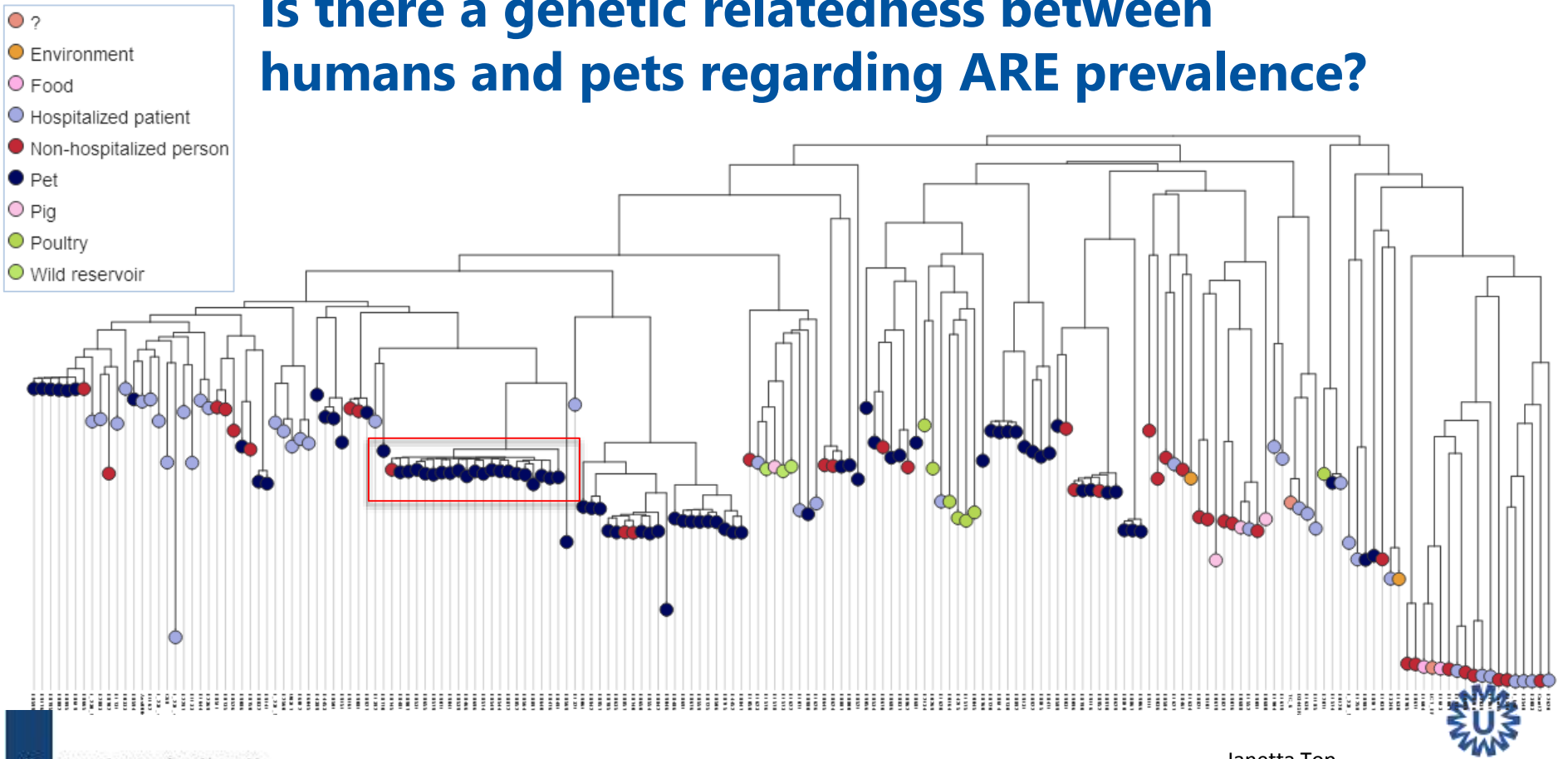
No co-colonization observed

Is there a genetic relatedness between humans and pets regarding ARE prevalence?

- Phylogenetic analysis based on core genome multilocus sequence typing (cgMLST)

Lebreton F, et al. *MBio*. 2013;4(4):1–10.

Is there a genetic relatedness between humans and pets regarding ARE prevalence?



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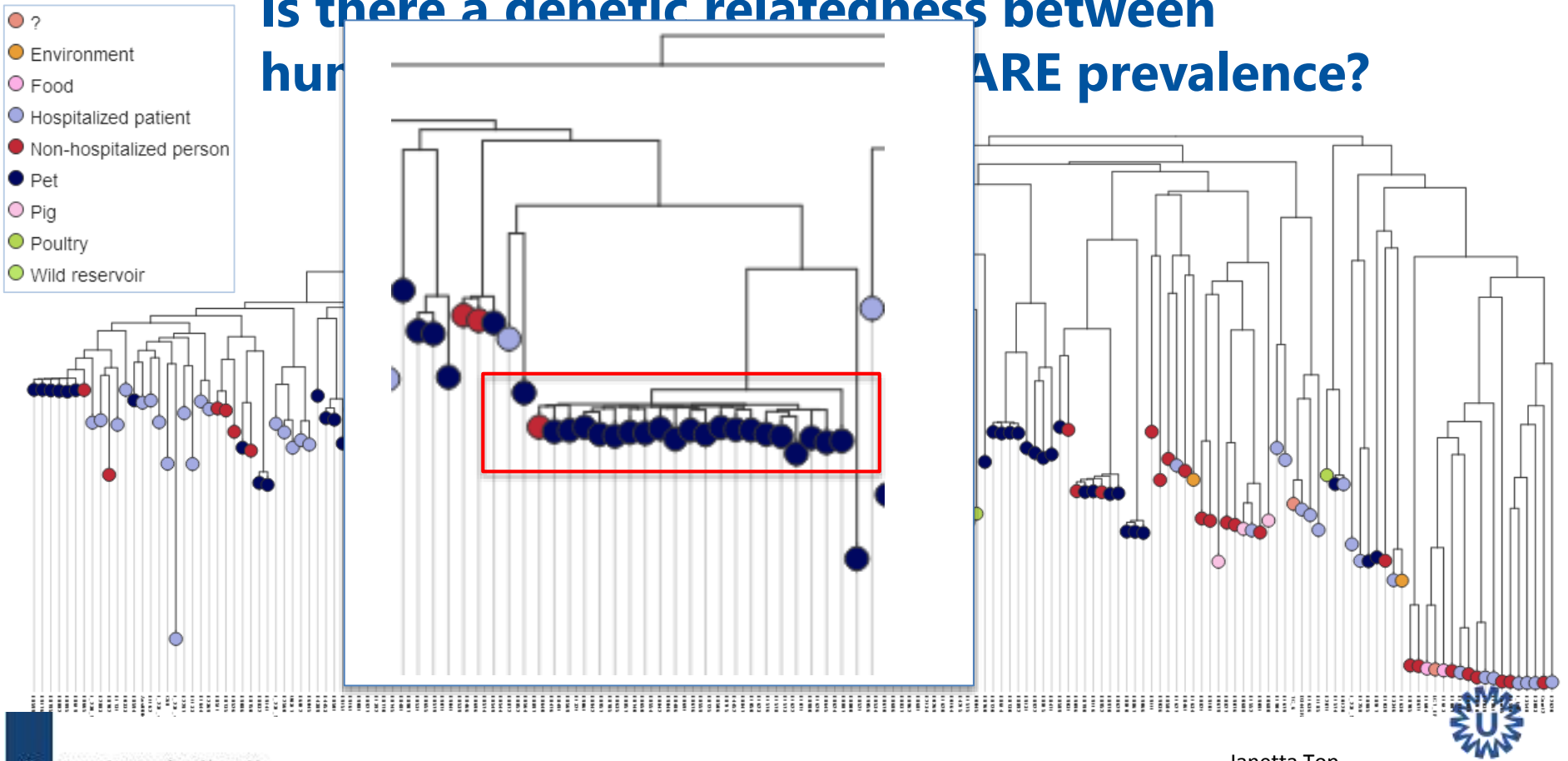
Is there a genetic relatedness between hur ARE prevalence?



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Discussion

- Is there a genetic relatedness between humans and pets regarding ARE prevalence?
  - Based on only epidemiological data: NO
    - No co-colonization within 1 household
    - Having a dog in the same household is not observed as a risk factor
  - Based on molecular cgMLST data: probably YES
    - Dogs and human ARE are co-located in the same phylogenetic lineages

Conclusion

- VRE only observed in 1 human and 2 dogs
- What about ARE?
  - Prevalence in human relatively low (1.5%)
  - High prevalence in dogs (25.6%)
  - antibiotic use and proton pump inhibitors are risk factors for ARE in humans
  - antibiotic use and eating raw meat are risk factors for ARE in dogs
  - No co-carriage in human-pet pairs
  - Human and pet isolates are molecular closely related

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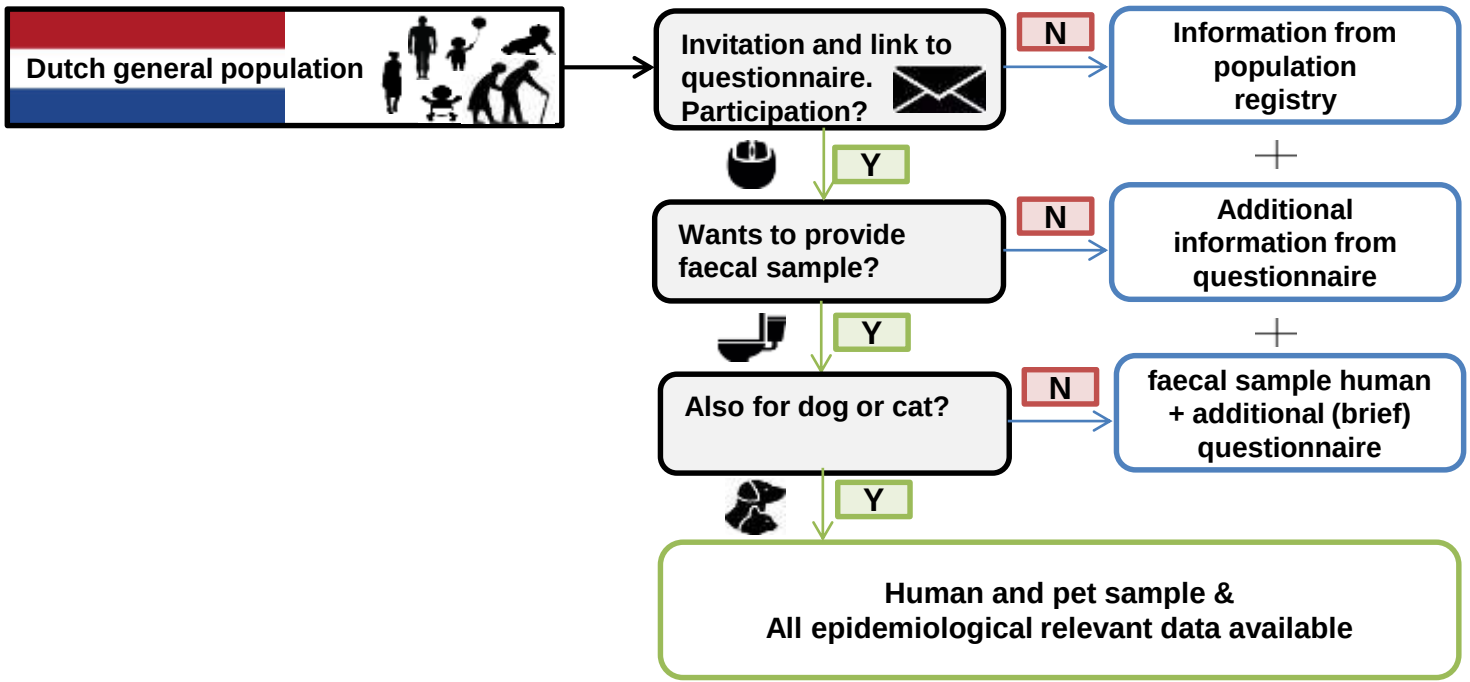
Thank you!

Epidemiological data collection:



Risk factors for ARE and VRE carriage in humans, dogs and cats

- Missing data → imputation
- Logistic regression:
  - Model for humans
  - Model for dogs
- P≤0.25 selected for multivariate models
- Multivariate analyses: forward stepwise regression analyses, model selection based on Akaike information criteria
- Covariates considered as risk factor if P value <0.05
- Model checked for confounders and interactions.
- Internal validation → bootstrapping

Variables	ARE+ (n=29) n (%)	ARE- (n=1963) n (%)
age		
<18	6/29 (20.7)	259/1962 (13.2)
≥18	23/29 (79.3)	1703/1962 (86.8)
level of education		
Intermediate/lower educated (degree below BSc.)	16/29 (55.2)	728/1938 (37.6)
High educated (BSc., MSc., PhD degree)	13/29 (44.8)	1210/1938 (62.4)
gender (male)	12/29 (41.4)	878/1962 (44.8)
country of birth is the Netherlands	28/29 (96.6)	1870/1937 (96.5)
use of AB in past 8 wk	7/27 (25.9)	128/1829 (7.0)
use of AB in past 6 m	9/29 (31.0)	322/1932 (16.7)
use of proton pump inhibitors in past 6 m	10/29 (34.5)	318/1932 (16.5)
use of blood pressure lowering drugs in past 6 m	8/29 (27.6)	379/1932 (19.6)
use of tranquillizers in past 6 m	1/29 (3.4)	72/1932 (3.7)
use of cholesterol lowering drugs in past 6 m	5/29 (17.2)	287/1932 (14.9)
use of antidiabetic drugs in past 6 m	2/29 (6.9)	68/1932 (3.5)
hospitalization in past 12 m	1/29 (3.4)	155/1957 (7.9)
hospitalization in past 4 wk	0/26 (0.0)	14/1824 (0.8)
hospitalization household member in past 12 m	5/29 (17.2)	196/1731 (11.3)
any complaints in past 6 m	17/29 (58.6)	1210/1897 (63.8)
travel abroad in past 12 m	16/29 (55.2)	1330/1950 (68.2)
travel abroad in past 4 wk	3/27 (11.1)	387/1824 (21.2)
animals in/around home	14/29 (48.3)	949/1943 (48.8)
dog in household	9/29 (31.0)	450/1943 (23.2)
cats in household	6/29 (20.7)	410/1943 (21.1)
direct contact animals in past 4 wk	13/27 (48.1)	1068/1807 (58.9)
ate raw or undercooked meat in past week	9/26 (34.6)	532/1818 (29.3)

	Dogs		Cats	
Variables	ARE+ (n=71) n (%)	ARE- (n=199) n (%)	ARE+ (n=6) n (%)	ARE- (n=118) n (%)
age				
<6	34/70 (48.6)	91/198 (46.0)	1/6 (16.7)	39/109 (35.8)
≥6	36/70 (51.4)	107/198 (54.0)	5/6 (83.3)	70/109 (64.2)
gender (male)	34/70 (48.6)	96/198 (48.5)	4/6 (66.7)	42/108 (38.9)
hospitalized or consult	14/62 (22.6)	36/189 (19.0)	0/6 (0.0)	11/96 (11.5)
AB in past 8 wk	4/61 (6.6)	14/189 (7.4)	1/6 (16.7)	7/95 (7.4)
AB in past 6 m	17/69 (24.6)	25/199 (12.6)	0/6 (0.0)	11/109 (10.1)
kennel in past 4 wk	5/62 (8.1)	16/189 (8.5)	0/6 (0.0)	1/96 (1.0)
any complaints in past 4 wk	18/59 (30.5)	56/186 (30.1)	3/5 (60.0)	35/95 (36.8)
abroad in past 12 m	14/70 (20.0)	31/200 (16.1)	0/6 (0.0)	0/108 (0.0)
contact other animals	33/69 (47.8)	90/193 (46.6)	3/6 (50.0)	68/108 (63.0)
catch prey	11/68 (16.2)	41/191 (21.5)	3/6 (50.0)	53/108 (49.1)
coprophagy	24/70 (34.3)	50/198 (25.3)	-	-
eat raw meat	18/70 (25.7)	17/199(8.5)	0/6 (0.0)	9/109 (8.3)
eat wet food	15/70 (21.4)	37/199 (18.6)	5/6 (83.3)	70/109 (64.2)
eat dry food	63/70 (90.0)	188/199 (94.5)	6/6 (100.0)	103/109 (94.5)

Discussion

- ARE from healthy humans do not cluster among *E. faecium* susceptible for ampicillin from the community
- No direct transmission (no co-carriage)
  - but close phylogenetic linkage suggests epidemiological linkage
- AB use is a risk factor for ARE in humans and dogs:
  - ARE already present -> Selection due to AB use -> detection?
  - In humans: continues exposure of ARE (due to dogs?) -> using AB -> colonization -> detection?

Limitations

- Relatively low number of carriers of ARE and VRE in humans and cats