Supporting information

Insights in livestock-related microbial concentrations in air at residential level in a livestock dense area

Myrna M.T. de Rooij*, Gerard Hoek¹, Heike Schmitt¹,², Ingmar Janse², Arno Swart², Catharina B.M. Maassen², Marjolijn Schalk², Dick J.J. Heederik¹, Inge M. Wouters¹

Affiliations

1. Institute for Risk Assessment Sciences (IRAS), Utrecht University, Yalelaan 2, 3584 CM Utrecht, The Netherlands
2. National Institute for Public Health and the Environment (RIVM), Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands

Total number of pages: 25
Pages on methods: 2 - 7
Number of tables: 6
Number of figures: 7
Supplementary methods

Note. qPCR analyses for *C. jejuni* have been performed on two qPCR devices. Differences between the devices have been taken into account.

| Gene target               | Gene coding for               | Primer(s) and probe sequences (5’-3’)                                      | Source/reference |
|---------------------------|-------------------------------|---------------------------------------------------------------------------|-----------------|
| *uidA* (*E. coli*)        | Beta-glucuronidase            | *uidA*-P1: GTG TGA TAT CTA CCC GCT TCG C                                  | Frahm et al., 2003 |
|                           |                               | *uidA*-P2: AGA ACG GTT TGT GGT TAA TCA GGA                                |                  |
|                           |                               | *uidA* Probe: ATTO425-TGC GCA TCC GTG TCA TCA TCA GCT TCG TCC             |                  |
|                           |                               |                                                                          |                  |
| *Tuf* (*Staphylococcus* spp.) | Elongation factor Tu   | *Tuf*-P1: AAACAACGTGTACCTGTTAGGAATAG                                        | Kilic et al., 2010 |
|                           |                               | *Tuf*-P2: AGTACGGAATAAATGTTG                                             |                  |
| *glyA* (*C. jejuni*)      | Serine hydroxymethyltransferase | *Cj*-F: TAATGTTCAGGCTTTACGTTGTTTCT                                       | Jensen et al., 2005 |
|                           |                               | *Cj*-R: GAAGAAACTTACTTTTGCACCATGA                                       |                  |
|                           |                               | *Cj*-P: ATTO425-AAATCAAGCCGCTATAAACCCACCTTGATTAGC-BHQ2                   |                  |
| *tetW*                    | Tetracycline resistance protein | *tetW*-P1: CGGCAGCGCAAGAGAGAAAC                                         | Walsh et al., 2011 |
|                           |                               | *tetW*-P2: CGGGTCAGTATCCGCCGAAGTT                                    |                  |
|                           |                               | *tetW* Probe: 6FAM-CTGGACGCTTTTACAG-BHQ1                                |                  |
| *mecA*                    | Altered penicillin-binding protein (PBP-2a) leading to β-lactam resistance | *MecA*-P1: AAATATTTATGCTGATTCGATTTGC                                      | Kilic et al., 2010 |
|                           |                               | *MecA*-P2: CGTTAAATGCTGATTTTCTAAT                                       |                  |
|                           |                               | *mecA* Probe: ATTO425-CAAGGTTAAATGCAAACCCAT-CAGA-BHQ2                   |                  |

| Gene target | Activation | Denaturation | Annealing/elongation | Cooling | Number of cycles |
|-------------|------------|--------------|-----------------------|---------|------------------|
| *E. coli*   | 95°C for 5 min | 94°C for 15 s | 60°C for 45 s         | 50°C for 30 s | 50 |
| *Staphylococcus* spp. | 95°C for 5 min | 94°C for 15 s | 60°C for 45 s         | 50°C for 30 s | 50 |
| *C. jejuni* | 95°C for 5 min | 95°C for 15 s | 60°C for 60 s         | 40°C for 30 s | 50 |
| *tetW*      | 95°C for 3 min | 95°C for 15 s | 59°C for 45 s         | NA      | 45              |
| *mecA*      | 95°C for 5 min | 94°C for 15 s | 60°C for 45 s         | 50°C for 30 s | 50 |
## Gene target | Youden index | Sensitivity | Specificity
---|---|---|---
*E. coli* | 0.86 | 0.91 | 0.95
*Staphylococcus* spp. | 0.69 | 0.70 | 0.99
*C. jejuni* Device I | NA | NA | NA
*C. jejuni* Device II | NA | NA | NA
tetW | 0.96 | 0.98 | 0.98
mecA | 1 | 1 | 1

## Gene target | Dilution series of standard | Content standard
---|---|---
*E. coli* | 10^0 to 10^-7 | 2.71 x 10^6 copies per µl
*Staphylococcus* spp. | 10^0 to 10^-7 | 1.50 x 10^6 copies per µl
*C. jejuni* Device I | 10^0 to 10^-6 | 1.33 x 10^2 copies per µl
*C. jejuni* Device II | 10^0 to 10^-6 | 1.33 x 10^2 copies per µl
tetW | 5^-4 to 5^-11 | 1.25 x 10^7 copies per µl
mecA | 5^-2 to 5^-11 | 2.52 x 10^6 copies per µl

## Gene target | LOD cycle | Calibration curve
---|---|---
*E. coli* | 37.4 | -2.71 * log_{10}(GEcopies) + 39.24
*Staphylococcus* spp. | 39.7 | -3.79 * log_{10}(GEcopies) + 46.37
*C. jejuni* Device I | 41.6 | -3.09 * log_{10}(GEcopies) + 37.98
*C. jejuni* Device II | 41.6 | -3.12 * log_{10}(GEcopies) + 38.05
tetW | 35 | -3.45 * log_{10}(copies) + 40.05
mecA | 38.5 | -3.19 * log_{10}(copies) + 38.05
Oligonucleotides for qPCR assays were designed using the software package Visual Oligonucleotide Modeling Platform version 6 (DNA software Inc. Ann Arbor, USA). A fragment of the gene encoding the blue fluorescent protein was used as a non-target control sequence. In order to produce a DNA fragment for spiking, a primer pair was designed for amplification of a 424 bp fragment from this gene (bfptrg-f/r). Amplification was carried out using the HotStar Taq Master Mix Kit (Qiagen, Westburg, the Netherlands) and 400 nM primers in a total reaction volume of 50 μl. Primer sets were designed using Visual OMP software Thermocycling conditions were as follows: 95°C for 15 min, 40 cycles at 95°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec, followed by a final step at 72°C for 7 min. Thermocycling reactions were carried out in a Px2 thermal cycler (Thermo Electron Corporation, Breda, the Netherlands). The resulting PCR product was quantified by using the Quantus fluorometer and the Qubit dsDNA HS Assay Kit (Promega).

For detection of this gene, a hydrolysis probe and primers were designed which amplify a 78 bp region within this bfp fragment. All qPCR reactions were carried out in a final volume of 20 μl containing iQ Multiplex Powermix (Bio-Rad, Veenendaal, the Netherlands), 200 nM of each primer and 100-300 nM hydrolysis probes and 3 μl of DNA template. The multiplex real-time qPCR assays had been designed for an optimal annealing temperature of 60°C and the thermal cycling conditions were as follows: First enzyme activation at 95°C for 5 min, followed by amplification and detection by 45 cycles at 95°C for 5 sec and 60°C for 35 sec. Measurements were carried out on a Lightcycler 480 (Roche, Almere, the Netherlands). Analyses were performed on the instruments software: LightCycler 480 Software release 1.5.0. SP3. Cq values were calculated using the second derivative method on the LightCycler using the DNA 1000 kit.
Model used for imputation of missings:

The imputation model consisted of a random effects model where readings on samples <LOD were accommodated by specifying a censored likelihood. The model parameters were estimated in a Bayesian framework, using only weakly informative priors. The model was fitted using MCMC algorithms as implemented in JAGS (Plummer, 2003).

The model equations for the random effects model are as follows:

For readings on samples taken at the reference site:

\[ Y_{r}[i,j] = \alpha_r + \mu_r[SEQ[i]] + \nu_r[i] + \varepsilon_r[j] \]

For readings on samples taken at the (non-reference) measurement sites:

\[ Y_{s}[i,j] = \alpha_s + \delta_s[LOC[i]] + \mu_s[SEQ[i]] + \nu_s[i] + \varepsilon_s[j] \]

where \( i \) indexes the samples (filters) and \( j \) indexes the readings. \( SEQ[i] \) is the measurement occasion and \( LOC[i] \) the location where a specific sample (indexed by \( i \)) was taken.

The parameters \( \alpha_r \) and \( \alpha_s \) represent the average readings at respectively the reference site and the (non-reference) measurement sites across measurement occasions. The random effect parameters are modelled as follows:

Random location-effect for samples taken at the (non-reference) measurement sites:

\[ \delta_s \sim N(0,\sigma^2_{\delta_s}) \]

Random measurement occasion-effects for samples taken at the reference site (\( \mu_r \)) and the (non-reference) measurement sites (\( \mu_s \)):

\[
\begin{bmatrix}
\mu_r \\
\mu_s
\end{bmatrix} \sim N\left(\begin{bmatrix} 0 \\
0 \end{bmatrix}, \begin{bmatrix} \sigma^2_{\mu_r} & \sigma_{\mu rs} \\
\sigma_{\mu rs} & \sigma^2_{\mu s} \end{bmatrix}\right)
\]

By specifying the random effects for the reference site and (non-reference) measurement sites to be correlated, we allow the temporal variability at the reference site to be linked to that at the (non-reference) measurement sites.

Random sample-effect for samples taken at the reference site (\( \nu_r \)) and the (non-reference) measurement sites (\( \nu_s \)):

\[ \nu_r \sim N(0,\sigma^2_{\nu r}) \]
\[ \nu_s \sim N(0,\sigma^2_{\nu s}) \]

The residuals for samples taken at the reference site (\( \varepsilon_r \)) and the (non-reference) measurement sites (\( \varepsilon_s \)):

\[ \varepsilon_r \sim N(0,\sigma^2_{\varepsilon r}) \]
\[ \varepsilon_s \sim N(0,\sigma^2_{\varepsilon s}) \]

where the \( \sigma^2 \) are the random effects variances and \( \sigma_{\mu rs} \) is the covariance between \( \mu_r \) and \( \mu_s \).
Structure of mixed models containing one livestock-related predictor

For Staph spp, E.coli, tetW and mecA:

$$Y_{t,s} \sim \beta_0 + u_s + \beta_i X_{i(spatial)} + \text{refC}_{(temporal)} + \epsilon$$

For C. jejuni:

$$\ln(P_{t,s}/(1-P_{t,s})) \sim \beta_0 + u_s + \beta_i X_{i(spatial)} + \text{refNP}_{(temporal)} + \epsilon$$

$Y$ = concentration in ln copies per m$^3$ / (time + site)

$P$ = probability of a positive sample / (time + site)

$\beta_0$ = intercept

$u_s$ = random effect for the intercept of site $s$

$\beta_i$ = regression coefficient for predictor

$X_i$ = livestock-related predictor of the surroundings (spatial), see Tables 1 and 2 and Supplementary Table S2 for complete overview of all predictors

$\text{refC}$ = measured concentration (ln copies per m$^3$) at reference site during that measurement period (temporal)

$\text{refNP}$ = sample outcome (negative or positive) measured at reference site during that measurement period (temporal)

$\epsilon$ = residual error
Structure of mixed models containing multiple livestock-related predictors

For Staph spp, E.coli, tetW and mecA:  
\[ Y_{t,s} \sim \beta_0 + u_s + \beta_1 X_1(\text{spatial}) + \beta_2 X_2(\text{spatial}) + \beta_3 X_3(\text{spatial}) + \beta_4 X_4(\text{spatial}) + \beta_5 X_5(\text{spatial}) + \beta_6 X_6(\text{spatial}) + \beta_7 X_7(\text{spatial}) + \text{refC}(\text{temporal}) + \epsilon \]

For C. jejuni:  
\[ \ln(P_{t,s}/(1-P_{t,s})) \sim \beta_0 + u_s + \beta_1 X_1(\text{spatial}) + \beta_2 X_2(\text{spatial}) + \beta_3 X_3(\text{spatial}) + \beta_4 X_4(\text{spatial}) + \beta_5 X_5(\text{spatial}) + \beta_6 X_6(\text{spatial}) + \beta_7 X_7(\text{spatial}) + \text{refNP}(\text{temporal}) + \epsilon \]

\( Y \) = concentration in Ln copies per m\(^3\)

\( P \) = probability of a positive sample

\( \beta_0 \) = intercept

\( u_s \) = random effect for the intercept of site s

\( \beta_i \) = regression coefficient for livestock-related predictor

\( \text{refC} \) = measured concentration (Ln copies per m\(^3\)) at reference site during that measurement period (temporal)

\( \text{refNP} \) = sample outcome (negative or positive) measured at reference site during that measurement period (temporal)

\( \epsilon \) is residual error

| Symbol | Predictor (spatial) expressed in summation weighted distance number of animals present at a farm (n animals /distance in m) for all farms in the buffer |
|--------|--------------------------------------------------------------------------------------------------|
| \( X_1 \) | N pigs weighted to distance in 3000m buffer (\( \Sigma(n/m) \)) |
| \( X_2 \) | N poultry animals weighted to distance in 3000m buffer (\( \Sigma(nm) \)) |
| \( X_3 \) | N cows weighted to distance in 3000m buffer (\( \Sigma(n/m) \)) |
| \( X_4 \) | N horses weighted to distance in 3000m buffer (\( \Sigma(n/m) \)) |
| \( X_5 \) | N goats weighted to distance in 3000m buffer (\( \Sigma(n/m) \)) |
| \( X_6 \) | N sheep weighted to distance in 3000m buffer (\( \Sigma(n/m) \)) |
| \( X_7 \) | N fur animals weighted to distance in 3000m buffer (\( \Sigma(N/m) \)) |

Supplementary information on the intercept by applying per gene target the above described model equation:

| Gene target | Intercept | 95% Confidence Interval | Random effects Intercept Variance |
|-------------|-----------|------------------------|----------------------------------|
| E.coli      | -0.315    | -0.895; 0.277          | 0.105                            |
| Staph spp   | -0.162    | -1.495; 1.162          | 0.403                            |
| tetW        | 0.754     | -0.281; 1.817          | 0.151                            |
| mecA        | -0.249    | -0.868; 0.370          | 0.296                            |
| C.jejunii   | -1.895    | -2.957; -0.896         | <0.001                           |
### Table S.1. Distribution of livestock characteristics of surroundings over 61 measurement sites

| Livestock characteristics of surroundings | 10th Percentile | Median | 90th Percentile |
|------------------------------------------|-----------------|--------|-----------------|
| Distance site to nearest livestock farm (-1 * m) | -795.573 | -296.270 | -103.231 |
| Distance site to nearest livestock farm (m^-1) | 0.001 | 0.003 | 0.010 |
| N livestock farms within 250m buffer | 0 | 0 | 2 |
| N of livestock farms within 500m buffer | 0 | 2 | 7 |
| N livestock farms within 1000m buffer | 2 | 10 | 20 |
| N livestock farms within 3000m buffer | 61 | 98 | 131 |
| N farms weighted to distance in a 1000m buffer (Σ(N/m)) | 0.002 | 0.018 | 0.041 |
| N farms weighted to distance in a 3000m buffer (Σ(N/m)) | 0.039 | 0.058 | 0.099 |
| N pig farms weighted to distance in a 1000m buffer (Σ(N/m)) | 0.000 | 0.003 | 0.014 |
| N pigs weighted to distance in a 1000m buffer (Σ(N/m)) | 0.000 | 9285.430 | 42021.233 |
| N poultry farms weighted to distance in a 1000m buffer (Σ(N/m)) | 0.000 | 0.002 | 0.009 |
| N poultry animals weighted to distance in a 1000m buffer (Σ(N/m)) | 0.000 | 25009.683 | 505513.511 |
| N cattle farms weighted to distance in a 1000m buffer (Σ(N/m)) | 0.000 | 0.007 | 0.014 |
| N cows weighted to distance in a 1000m buffer (Σ(N/m)) | 0.000 | 1328.948 | 4104.613 |
| N horse farms weighted to distance in a 1000m buffer (Σ(N/m)) | 0.000 | 0.002 | 0.006 |
| N horses weighted to distance in a 1000m buffer (Σ(N/m)) | 0.000 | 66.158 | 217.874 |
| N pig farms weighted to distance in a 3000m buffer (Σ(N/m)) | 0.008 | 0.015 | 0.034 |
| N pigs weighted to distance in a 3000m buffer (Σ(N/m)) | 54119.940 | 134540.853 | 325480.019 |
| N poultry farms weighted to distance in a 3000m buffer (Σ(N/m)) | 0.001 | 0.007 | 0.015 |
| N poultry animals weighted to distance in a 3000m buffer (Σ(N/m)) | 127718.993 | 1074477.054 | 3042530.271 |
| N cattle farms weighted to distance in a 3000m buffer (Σ(N/m)) | 0.016 | 0.026 | 0.038 |
| N cows weighted to distance in a 3000m buffer (Σ(N/m)) | 9166.616 | 18556.196 | 37897.834 |
| N horse farms weighted to distance in a 3000m buffer (Σ(N/m)) | 0.005 | 0.008 | 0.013 |
| N horses weighted to distance in a 3000m buffer (Σ(N/m)) | 303.632 | 950.831 | 1912.307 |
| N goat farms weighted to distance in a 3000m buffer (Σ(N/m)) | 0.000 | 0.000 | 0.002 |
| N goats weighted to distance in a 3000m buffer (Σ(N/m)) | 0.000 | 562.010 | 11834.215 |
| N sheep farms weighted to distance in a 3000m buffer (Σ(N/m)) | 0.000 | 0.000 | 0.003 |
| N sheep weighted to distance in a 3000m buffer (Σ(N/m)) | 346.152 | 710.223 | 2208.872 |
| N fur animal farms weighted to distance in a 3000m buffer (Σ(N/m)) | 0.000 | 0.001 | 0.002 |
| N fur animals weighted to distance in a 3000m buffer (Σ(N/m)) | 0.000 | 3990.745 | 31937.273 |
Table S.2.
Distribution of outcomes (above or below LOD) within samples collected at 61 measurement sites

| Target - in triplo tested | Above LOD | Summary |
|---------------------------|-----------|---------|
|                          | 0 out of 3| 1 out of 3| 2 out of 3| 3 out of 3| % at least 1 out of 3 |
| *E. coli*                 | 58        | 54      | 41       | 82        | 75%                  |
| *Staphylococcus* spp      | 11        | 10      | 24       | 190       | 95%                  |
| *C. jejuni*               | 136       | 56      | 31       | 12        | 42%                  |
| *mecA*                    | 29        | 33      | 39       | 134       | 88%                  |

| Target - in duplo tested  | Above LOD | Summary |
|---------------------------|-----------|---------|
|                          | 0 out of 2| 1 out of 2| 2 out of 2| % at least 1 out of 2 |
| *tetW*                   | 11        | 8       | 216     | 95%                 |
Table S.3.

Overview of Pearson correlations between endotoxin concentration, PM10 mass concentration; respectively, and gene targets DNA concentrations for concentrations over time measured at reference site (temporal correlation) and temporal adjusted site-average concentrations for the 61 measurement sites (spatial correlation)

|            | Temporal correlation | Spatial correlation |
|------------|----------------------|--------------------|
|            | Endotoxin | PM10 | Endotoxin | PM10 |
| *E. coli*  | 0.23      | 0.32 | 0.55      | 0.30 |
| *Staph spp* | 0.21      | 0.21 | 0.45      | 0.21 |
| tetW       | 0.23      | 0.21 | 0.57      | 0.30 |
| *mecA*     | 0.14      | 0.28 | 0.52      | 0.32 |

Note. Concentrations expressed in ln copies/m^3 for gene targets, ln EU/m^3 for endotoxin, and ln µg/m^3 for PM10
Table S.4.

Univariable associations of animal species-specific livestock characteristics with measured airborne bacteria and AMR genes based on mixed modelling

| Variables (scaled 10^{th}-90^{th} percentile) | Commensal bacteria (ln copies/m^3) | AMR genes (ln copies/m^3) | Pathogenic bacteria (0/1) |
|----------------------------------------------|------------------------------------|---------------------------|--------------------------|
|                                              | E. coli               | Staph spp | tetW | mecA | C. jejuni |
| Ratio | CI LB  | CI UB | Ratio | CI LB | CI UB | Ratio | CI LB | CI UB | Ratio | CI LB | CI UB | Ratio | CI LB | CI UB |
| N pig farms weighted to distance in 1000m buffer | 2.10* | 1.52 | 2.92 | 1.73 | 0.92 | 3.29 | 1.97* | 1.36 | 2.86 | 1.72* | 1.02 | 2.89 | 2.33* | 1.21 | 4.81 |
| N pigs weighted to distance in 1000m buffer | 2.34* | 1.70 | 3.22 | 2.32* | 1.22 | 4.35 | 2.16* | 1.49 | 3.13 | 2.05* | 1.22 | 3.46 | 2.34* | 1.2 | 4.84 |
| N poultry farms weighted to distance in 1000m buffer | 1.52* | 1.01 | 2.29 | 1.72 | 0.84 | 3.53 | 1.70* | 1.09 | 2.61 | 2.29* | 1.31 | 4.01 | 1.81 | 0.86 | 4.00 |
| N poultry animals weighted to distance in 1000m buffer | 1.82* | 1.17 | 2.80 | 2.14 | 0.99 | 4.62 | 2.08* | 1.31 | 3.29 | 3.10* | 1.72 | 5.58 | 3.04* | 1.39 | 7.28 |
| N cattle farms weighted to distance in 1000m buffer | 1.52* | 1.02 | 2.29 | 2.32* | 1.15 | 4.62 | 1.46 | 0.93 | 2.29 | 1.60 | 0.89 | 2.86 | 2.39* | 1.13 | 5.53 |
| N cows weighted to distance in 1000m buffer | 1.93* | 1.31 | 2.89 | 2.46* | 1.22 | 5.00 | 1.73* | 1.12 | 2.69 | 2.12* | 1.19 | 3.78 | 2.99* | 1.43 | 6.94 |
| N horse farms weighted to distance in 1000m buffer | 1.48* | 1.02 | 2.14 | 1.90 | 1.00 | 3.60 | 1.57* | 1.06 | 2.34 | 1.52 | 0.90 | 2.59 | 1.42 | 0.72 | 2.92 |
| N horses weighted to distance in 1000m buffer | 1.46 | 0.97 | 2.18 | 2.36* | 1.19 | 4.71 | 1.58* | 1.03 | 2.46 | 1.54 | 0.86 | 2.77 | 1.34 | 0.63 | 2.97 |
|                       | N pig farms weighted to distance in 3000m buffer | N pigs weighted to distance in 3000m buffer | N poultry farms weighted to distance in 3000m buffer | N poultry animals weighted to distance in 3000m buffer | N cattle farms weighted to distance in 3000m buffer | N cows weighted to distance in 3000m buffer | N horse farms weighted to distance in 3000m buffer | N horses weighted to distance in 3000m buffer | N goat farms weighted to distance in 3000m buffer | N goats weighted to distance in 3000m buffer | N sheep farms weighted to distance in 3000m buffer |
|-----------------------|------------------------------------------------|--------------------------------------------|--------------------------------------------------|--------------------------------------------------|-----------------------------------------------|-----------------------------------------------|----------------------------------------------|-----------------------------------------------|------------------------------------------------|-----------------------------------------------|-----------------------------------------------|
|                       | 2.66* c                                        | 3.16* a                                    | 2.10*                                            | 2.51*                                            | 1.57*                                         | 2.69* b                                        | 1.86*                                        | 1.49                                           | 1.00                                          | 1.20                                          | 1.28                                           |
|                       | 1.93                                            | 2.23                                         | 1.38                                             | 1.65                                             | 1.05                                          | 1.75                                           | 1.23                                         | 0.92                                           | 0.68                                          | 0.79                                          | 0.81                                           |
|                       | 3.67                                            | 4.48                                         | 3.19                                             | 3.42* c                                         | 2.36*                                         | 7.03                                           | 2.77                                         | 2.41                                           | 1.48                                          | 1.80                                          | 2.03                                           |
|                       | 2.36*                                           | 3.42* c                                     | 3.39*                                            | 4.01* b                                         | 2.94* c                                      | 2.14                                           | 1.46                                         | 2.69*                                           | 1.51                                          | 1.00                                          | 1.77                                           |
|                       | 1.22                                            | 1.65                                         | 1.63                                             | 1.88                                             | 1.46                                         | 4.97                                           | 2.77                                         | 1.19                                           | 0.79                                          | 1.00                                          | 0.80                                           |
|                       | 4.62                                            | 7.03                                         | 7.10                                             | 8.67                                             | 5.93                                         | 2.44* c                                        | 5.93                                         | 6.11                                           | 2.92                                          | 3.97                                          | 3.86                                           |
|                       | 2.29* b                                         | 2.69*                                        | 2.36*                                            | 2.80* a                                         | 1.99*                                        | 1.49                                           | 1.99*                                        | 1.65                                           | 1.07                                          | 3.97                                          | 1.25                                           |
|                       |                                                 | 1.75                                         | 1.51                                             | 2.44* c                                         | 1.28                                         | 3.97                                           | 3.10                                         | 0.98                                           | 0.71                                          | 1.97                                          | 0.76                                           |
|                       |                                                 | 4.10                                         | 3.25                                             | 3.90* c                                         | 1.23                                         | 3.72                                           | 2.20*                                        | 2.77                                           | 1.62                                          | 3.25                                          | 1.07                                           |
|                       |                                                 | 3.25*                                        | 2.27                                             | 5.10* a                                         | 3.97                                         | 7.24                                           | 3.20*                                        | 1.63                                           | 3.02                                          | 5.69* a                                       | 1.23                                           |
|                       |                                                 | 3.64* b                                     | 6.89                                             | 5.69* a                                         | 1.52                                         | 3.58* c                                        | 5.75                                         | 8.61                                           | 3.56                                          | 1.56                                          | 3.81                                           |
|                       |                                                 | 2.19*                                        | 3.56                                             | 5.63                                             | 2.59                                         | 14.11                                          | 3.35                                         | 3.63                                           | 3.02                                          | 3.82                                          | 11.01                                          |
|                       |                                                 | 1.33                                         | 2.66*                                            | 2.66*                                            | 1.69                                         | 5.81                                           | 3.86                                         | 5.63                                           | 5.81                                          | 1.01                                          | 5.63                                           |

Note: The table entries are not directly translatable to plain text. The values in the table are numerical and represent some form of statistical or measured data, possibly related to distances and weighted counts of various farm types within a 3000m buffer.
|                                | 1.36 | 0.96 | 1.95 | 1.72 | 0.94 | 3.16 | 1.20 | 0.81 | 1.77 | 1.62 | 0.98 | 2.66 | 1.78 | 0.94 | 3.6 |
|--------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|-----|
| N sheep weighted to distance in 3000m buffer |      |      |      |      |      |      |      |      |      |      |      |      |      |      |     |
| N fur animal farms weighted to distance in 3000m buffer | 1.70* | 1.09 | 2.66 | 2.20* | 1.02 | 4.76 | 1.73* | 1.07 | 2.80 | 1.72 | 0.90 | 3.25 | 1.36 | 0.59 | 3.25 |
| N fur animals weighted to distance in 3000m buffer | 1.65* | 1.08 | 2.51 | 1.92 | 0.92 | 4.01 | 1.60* | 1.01 | 2.53 | 1.73 | 0.95 | 3.19 | 1.48 | 0.67 | 3.39 |

Note. Mixed modelling was performed per animal species-specific livestock characteristic for each outcome. Associations were adjusted for temporal variation by taking into account concentrations measured at reference site.

Exponent of estimate = estimate of association exponentiated to represent ratio difference in concentration
OR = odds ratio
CI LB/CI UB = 95% confidence interval; lower bound, upper bound; respectively
Predictor variables truncated to 95th percentile and then scaled to 10-90 percentile range, thus predictor values divided by 10-90 percentile range of that predictor
Unit of variables (Σ(N/m))
Animal species included in table above meet the criteria set for each predictor of minimally one-third of sites having a nonzero value (thus animal species not included do not)
Marked a then strongest association for that gene target
Marked b then second strongest association for that gene target
Marked c then third strongest association for that gene target
Marked * then P-value < 0.05
Table S.5.

Results of sensitivity analyses of multivariable modelling of airborne bacteria and AMR genes excluding the livestock characteristic with highest VIF: distance weighted number of cows

| Variables (scaled 10th-90th percentile) | Commensal bacteria (in copies/m³) | AMR genes (in copies/m³) | Pathogenic bacteria (0/1) |
|----------------------------------------|---------------------------------|-------------------------|--------------------------|
|                                        | E. coli                         | Staph spp                | tetW                     | mecA                     | C. jejuni                   |
|                                        | Ratio  | CI LB  | CI UB  | Ratio  | CI LB  | CI UB  | Ratio  | CI LB  | CI UB  | Ratio  | CI LB  | CI UB  | OR   | CI LB  | CI UB  |
| N pigs weighted to distance in 3000m buffer | 2.77* | 1.77   | 4.31   | 2.05   | 0.83   | 5.10   | 2.14* | 1.28   | 3.60   | 1.97   | 1.00   | 3.86   | 2.31 | 0.82   | 6.59   |
| N poultry animals weighted to distance in 3000m buffer | 1.72* | 1.11   | 2.64   | 2.61* | 1.07   | 6.30   | 2.34* | 1.40   | 3.86   | 3.97* | 2.05   | 7.69   | 4.84* | 1.79   | 14.04  |
| N horses weighted to distance in 3000m buffer | 1.34  | 0.83   | 2.16   | 2.39   | 0.90   | 6.36   | 1.49  | 0.85   | 2.61   | 1.40   | 0.68   | 2.92   | 0.84 | 0.28   | 2.60   |
| N goats weighted to distance in 3000m buffer | 0.82  | 0.58   | 1.15   | 0.99   | 0.50   | 1.99   | 1.06  | 0.63   | 1.80   | 1.17   | 0.58   | 2.36   | 0.92 | 0.31   | 2.69   |
| N sheep weighted to distance in 3000m buffer | 1.11  | 0.69   | 1.75   | 1.20   | 0.47   | 3.03   | 0.71  | 0.48   | 1.06   | 0.79   | 0.47   | 1.32   | 0.78 | 0.35   | 1.77   |
| N fur animals weighted to distance in 3000m buffer | 1.08  | 0.76   | 1.52   | 1.36   | 0.68   | 2.75   | 1.15  | 0.77   | 1.70   | 1.23   | 0.73   | 2.08   | 0.92 | 0.41   | 2.01   |

Note. Multivariable mixed modelling was performed for each gene target. Models included six animal species together, associations were adjusted for temporal variation by taking into account concentrations measured at reference site.
Predictor variables truncated to 95th percentile and then scaled to 10-90 percentile range, thus predictor values divided by 10-90 percentile range of that predictor
Unit of variables (Σ(N/m))
Marked * then P-value < 0.05
VIF for all predictors < 3
Table S.6.

Results of sensitivity analyses of multivariable modelling of DNA concentrations of *Staphylococcus* spp studying the influence of excluding distance weighted number of pigs (Model A) and distance weighted number of poultry animals (Model B) on reported associations (Model R)

| Variables (scaled 10th-90th percentile) | Model A | Model B | Model R |
|----------------------------------------|---------|---------|---------|
|                                        | Ratio   | CI LB   | CI UB   | Ratio   | CI LB   | CI UB   | Ratio   | CI LB   | CI UB   |
| N pigs weighted to distance in 3000m buffer | NA      | NA      | NA      | 2.41    | 0.81    | 7.17    | 1.90    | 0.64    | 5.58    |
| N poultry animals weighted to distance in 3000m buffer | 2.86*   | 1.16    | 6.96    | NA      | NA      | NA      | 2.53*   | 1.03    | 6.23    |
| N cows weighted to distance in 3000m buffer | 1.93    | 0.57    | 6.49    | 1.68    | 0.39    | 7.10    | 1.21    | 0.29    | 5.10    |
| N horses weighted to distance in 3000m buffer | 2.51    | 0.91    | 6.89    | 1.75    | 0.64    | 4.76    | 2.32    | 0.84    | 6.30    |
| N goats weighted to distance in 3000m buffer | 0.98    | 0.45    | 2.14    | 0.93    | 0.42    | 2.05    | 1.17    | 0.45    | 3.03    |
| N sheep weighted to distance in a 3000m buffer | 0.91    | 0.38    | 2.16    | 1.62    | 0.64    | 4.06    | 0.95    | 0.44    | 2.05    |
| N fur animals weighted to distance in a 3000m buffer | 2.86    | 1.16    | 6.96    | 1.26    | 0.59    | 2.69    | 1.32    | 0.64    | 2.77    |

Note. Multivariable mixed model in spatial characteristics, associations adjusted for temporal variation by taking into account outcomes measured at reference site. Predictor variables truncated to 95th percentile and then scaled to 10-90 percentile range, thus predictor values divided by 10-90 percentile range of that predictor. Unit of variables (Σ(N/m)) Marked * then P-value < 0.05 VIF for all predictors < 4
Supplementary figures

Figure S.1.
Geographical distribution of the 61 measurement sites in a livestock dense area in the Netherlands covering the geographical spread of the health study areas. The figure was generated using ArcGIS (version 10.2.2; Esri) and the gray background was sourced from Esri Nederland & Community Maps Contributors.
Figure S.2.

Distribution of DNA concentrations (copies/m³) of livestock-related commensal bacteria and AMR-genes measured.
Figure S.3.
Correlation plots of concentrations of sampled collected in parallel at reference site (Panel A: *E. coli*, Panel B: *Staphylococcus* spp., Panel C: *tetW*, Panel D: *mecA*)

|                | Pearson correlation (P-value) | Spearman correlation (P-value) | Mean Coefficient of Variation |
|----------------|-----------------------------|--------------------------------|-------------------------------|
| *E. coli*      | 0.94 (<0.001)              | 0.89 (<0.001)                  | 5.5%                          |
| *Staph spp*    | 0.97 (<0.001)              | 0.93 (<0.001)                  | 5.3%                          |
| *tetW*         | 0.94 (<0.001)              | 0.91 (<0.001)                  | 2.7%                          |
| *mecA*         | 0.93 (<0.001)              | 0.84 (<0.001)                  | 15%                           |
Figure S.4.
Overview of repeatedly measured bio-aerosols concentrations at each measurement site; left column not adjusted for temporal variation, right column adjusted for temporal variation (Panel A: *E. coli*, Panel B: *Staphylococcus* spp., Panel C: *tetW*, Panel D: *mecA*)
Not adjusted for temporal variation

Adjusted for temporal variation
Note. Sites classification based on distance from site to nearest farm (less than 250m, between 250-500m, between 500-1000m, and further than 1000m).
Boxplots are ranked based on distance from site to nearest farm; from left to right increase in distance to nearest farms.
Dual y-axes; left y-axis concentrations expressed as ln copies/m$^3$, right y-axis concentrations expressed as copies/m$^3$.
Figure S.5.
Correlation plots of concentrations of sampled collected in parallel at reference site (Panel A: *E. coli*, Panel B: *Staphylococcus* spp., Panel C: tetW, Panel D: mecA)

|          | Pearson correlation | Spearman correlation | Mean Coefficient of Variation |
|----------|---------------------|----------------------|-------------------------------|
| *E. coli*| 0.94 (<0.001)       | 0.89 (<0.001)        | 5.5%                          |
| *Staphylococcus* spp. | 0.97 (<0.001)       | 0.93 (<0.001)        | 5.3%                          |
| tetW     | 0.94 (<0.001)       | 0.91 (<0.001)        | 2.7%                          |
| mecA     | 0.93 (<0.001)       | 0.84 (<0.001)        | 15%                           |
Figure S.6.
Temporal relation between DNA concentrations (ln copies/m^3) of the gene targets measured over time at the reference site

Note. Red line is based on LOESS smoothed fit.
*** = P-value < 0.001
Figure S.7.

Pearson correlation matrix of livestock determinants describing weighted distance animal numbers and weighted distance farm numbers.
