Supplementary material for:
Socially engaged calves are more likely to be colonised by VTEC O157:H7 than individuals showing signs of poor welfare

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Supplementary Table S1. Description of indicators used for welfare assessment of the calves.

| Individual assessment | Definition: | Based on: |
|-----------------------|-------------|----------|
| **Body Condition Score** | Palpation and scoring from 1 (very thin) to 5 (fat) based on the amount of muscle/fat covering over the lumbar vertebrae’s and hips bones. Split into Poor (score 1-2) and Normal (3-4). No score 5 was observed. | 1 |
| **Cleanliness** | | 2-4 |
| Below hocks | Ocular observation and scoring from 1 to 3: No dirt; Minor splashing of manure (smaller than a hand in total); Distinct dried plaques of manure (larger than a hand) or 50% of area covered in wet manure | |
| Upper hind legs | | |
| **Body Coat condition** | Ocular observation and scoring: Poor (dull, shaggy or long rough hairs); Normal (shiny and short hairs) | 3 |
| **Conjunctivitis** | Ocular observation of inflammation (redness/swelling) around the eye | 5 |
| **Fearfulness** | Freestanding calf is approached with a speed of 1 step per second with experimenter aiming at reaching shoulder level. Stop at a distance of one arm’s length and arm is lifted to touch the animal. Distance when avoidance reaction occurs noted. Scores 1 to 5: >2 m, <2m, <1m, withdrawal when arm is lifted or if the animal can be touched (for at least 2s) were collapsed to scores 1 to 3: >2 m, <2m, <1m. | |
| **Faecal consistency** | Ocular observation and assessment of consistency. Scored as: Diarrhoea (faeces watery or slimy); Loose (faeces smooth and homogenous); Firm (faeces firm and break up) | 6 |
| **Hairless patches** | Ocular observation of number of areas >2 cm² with hair loss, extensive thinning of the coat due to parasites or hyperkeratosis with non-damaged skin were calculated. Up to 20 areas were counted. One area larger than the size of a hand counted as 20 small areas. | 3 |
| **Lameness** | Ocular observation and scoring from 1-3: No lameness; Uneven temporal rhythm; Reluctance to bear weight on a leg | 3 |
| **Nasal discharge** | Ocular observation of flow/discharge from the nostrils | 3 |
| **Number of wounds/inflammation** | As described above for Hairless patches but observation and counting of areas with damaged skin either in form of a scab or a wound, dermatitis due to ectoparasites or ear lesions due to torn off ear tags | 3 |
| **Reactivity** | Assessed during restraining for hair-sampling. Animal scored from 1-4 after release: 1. Animal is relaxed and not trying to resist being restrained. Curious and/or contact-seeking toward persons performing the sampling; 2. Animal is nervous and stepping around during sampling. May try to avoid sampling by moving the hind and lifting hind legs although not kicking; 3. Animal is forcefully trying to avoid being restrained by trying to escape or forcefully kicks; 4. Animal is paralyzed by the restraining. Increased respiration rate and introvert behaviour. No forceful attempts to escape or react to the sampling | 6 |
| **Ruminal fill*†** | Ocular observation and scoring as: Poor (visible triangle formed in left para lumbar fossa and small rumen¹); Normal (filled rumen is visible in Para lumbar fossa) | |
| **Size within group** | Ocular observation of group and scoring as: Small (calf is smaller than the average size in the group); Normal (calf size does not differ from other animals); Large (calf is larger than the average size in the group) | |
| **Tearing (epiphora)** | Ocular observation of discharge from the eyes. Estimated length of tearstain noted | |
| **Butting** | Animal pushes front head against other calf but receiving animal does not move | 7 |
| **Coughing** | Expelling air from the lungs with a sudden sharp sound. | 8 |
| **Cross-sucking** | Calf performs sucking movements when having part of another calf’s body in its mouth. Could also include sucking at prepuce or testis | 7 |
| **Displacement** | Animal displaces another calf by pushing forehead or base of head against other calf and thereby forcing it to move | 7 |
| ** Fighting** | Two calves butting heads standing front to front. | 9 |
| **Headshake** | Shaking or rotating head | 9 |
| **Licking other calf** | Any part of other calf is licked one or multiple times without pause | 7 |
| Locomotor play                                      | Running, turning, jumping, bucking or play with environment (for example playful butting of objects/pen)  |
|----------------------------------------------------|---------------------------------------------------------------------------------------------------------|
| Mounting                                           | Calf mounts another calf from any direction                                                           |
| Oral manipulation of environment (e.g. pen, floor) | Licking, nibbling or suckling objects or environment                                                  |
| Rubbing/scratching                                 | Repeatedly rubbing claw of hind leg over head, neck shoulder or moving body against objects/wall/other calf |
| Self-licking                                       | Licking any part of own body                                                                           |
| Stretching                                         | Stretching back, tail, hind legs or neck separately or simultaneously                                  |
| Tongue playing/rolling                            | Swaying, turning and partly rolling and unrolling the tongue inside the open mouth or when tongue is extended |

*Calves<2 weeks of age given milk – all assessed as normal.
†Frequency of behaviours observed during 20 minutes per pen.

References:

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### Supplementary Table S2

Descriptive statistics and univariable analysis of variables included in the analysis. Quantitative variables are presented in italics with average in group and range (minimum and maximum). Univariable analysis shows results from a logistic regression model with colonisation status (as detected by rectoanal mucosal swabs) as response variable. The presented variable and pen (as random effect to account for clustering) were included as explanatory variables. Logistic regression was performed in R using the package lme4.

| Status VTEC O157:H7 | Univariable analysis |
|---------------------|----------------------|
|                     | Non-colonised n=135  | Colonised n=56 | OR (95% conf int) | p-value |
| Age (days)          | Average (min – max)  |                |                  |         |
|                     | 138.8 (19-302)       | 113.4 (19-255) | 0.993 (0.99-1.00) | 0.037   |
| Sex                 |                      |                |                  |         |
| Female              | 88                   | 47             | 0.43 (0.15-1.09) | 0.037   |
| Male                | 30                   | 7              | 0.18 (0.03-0.79) |         |
| Castrated male      | 17                   | 2              |                  |         |
| Missing             | 0                    | 0              |                  |         |
| Size                |                      |                |                  |         |
| Normal              | 82                   | 40             | 0.44 (0.15-1.17) | 0.28    |
| Small               | 29                   | 6              |                  |         |
| Large               | 23                   | 10             | 0.92 (0.36-2.23) |         |
| Missing             | 1                    | 0              |                  |         |
| BCS                 |                      |                |                  |         |
| Normal (3-5)        | 103                  | 47             | 0.55 (0.21-1.34) | 0.21    |
| Poor (1-2)          | 31                   | 8              |                  |         |
| Missing             | 1                    | 1              |                  |         |
| Cleanliness below hocks |                |                |                  |         |
| 1                   | 31                   | 19             | 0.57 (0.24-1.27) | 0.11    |
|                     | 86                   | 33             |                  |         |
| 2                   | 61                   | 22             | 0.67 (0.33-1.35) |         |
|                     | 15                   | 2              |                  |         |
| 3                   | 14                   | 3              | 0.40 (0.08-1.46) |         |
|                     | 5                    | 2              |                  |         |
| Cleanliness body    |                      |                |                  |         |
| 1                   | 55                   | 29             | 0.31             |         |
|                     | 61                   | 22             |                  |         |
| 2                   | 14                   | 3              |                  |         |
|                     | 5                    | 2              |                  |         |
| Cleanliness upper hind |                |                |                  |         |
| 1                   | 23                   | 14             | 0.36             |         |
|                     | 73                   | 28             |                  |         |
| 2                   | 36                   | 12             | 0.50 (0.18-1.38) |         |
|                     | 3                    | 2              |                  |         |
| Coat condition      |                      |                |                  |         |
| Missing             | 3                    | 2              |                  |         |
| Normal              | 82                   | 38             | 0.71 (0.33-1.44) | 0.35    |
| Abnormal            | 50                   | 17             |                  |         |
| Missing             | 3                    | 1              |                  |         |
| None                | 76                   | 36             | 0.62 (0.28-1.33) | 0.44    |
| Hairless spots      |                      |                |                  |         |
| Few                 | 43                   | 14             |                  |         |
| Variable                        | No | Yes | Missing | Average | SD | 95% CI       |
|--------------------------------|----|-----|---------|---------|----|--------------|
| **Inflammation (wounds/swelling)** |    |     |         | 0.62    |    | (0.16-1.99)  |
| Missing                        | 2  | 1   |         |         |    |              |
| No                             | 115| 51  |         | 0.18    |    |              |
| Yes                            | 18 | 4   |         | 0.42    |    | (0.10-1.36)  |
| **Tearing (epiphora)**         |    |     |         | 0.66    |    |              |
| Missing                        | 2  | 1   |         |         |    |              |
| 0-2 cm                         | 82 | 35  |         | 0.99    |    | (0.44-2.18)  |
| 2-5 cm                         | 33 | 14  |         | 0.60    |    | (0.17-1.73)  |
| > 5 cm                         | 19 | 6   |         | 0.42    |    |              |
| **Nasal discharge**            |    |     |         | 0.41    |    |              |
| Missing                        | 1  | 1   |         |         |    |              |
| No                             | 83 | 30  |         | 1.35    |    | (0.66-2.73)  |
| Yes                            | 50 | 25  |         |         |    |              |
| **Fecal consistency**          |    |     |         | 0.09    |    |              |
| Loose                          | 81 | 37  |         | 1.03    |    | (0.47-2.16)  |
| Firm                           | 34 | 17  |         |         |    |              |
| Abnormal (diarrhoea)           | 20 | 1   |         | 0.1     |    | (0.01-0.54)  |
| Missing                        | 0  | 1   |         |         |    |              |
| **Distance avoidance test**    |    |     |         | 0.62    |    |              |
| 1-2m                           | 44 | 18  |         | 0.81    |    | (0.32-1.99)  |
| > 2m                           | 36 | 13  |         | 0.81    |    | (0.32-1.97)  |
| < 1m (incl. touch and withdrawal when arm is lifted) | 37 | 13 |         | 1.85 | | (0.48-6.91) |
| Missing by design              | 10 | 8   |         |         |    |              |
| Missing (at random)            | 8  | 4   |         |         |    |              |
| Low                            | 60 | 24  |         | 1.31    |    | (0.64-2.70)  |
| Average                        | 49 | 25  |         | 0.63    |    | (0.21-1.77)  |
| High                           | 26 | 6   |         |         |    |              |
| Missing                        | 0  | 1   |         |         |    |              |
| **Reactivity**                 |    |     |         | 0.37    |    |              |
| Average                        | 49 | 25  |         | 1.31    |    | (0.64-2.70)  |
| High                           | 26 | 6   |         | 0.63    |    | (0.21-1.77)  |
| Missing                        | 0  | 1   |         |         |    |              |
| **Cough**                      |    |     |         | 0.99    |    |              |
| No                             | 97 | 39  |         | 1.00    |    | (0.46-2.07)  |
| Yes                            | 37 | 16  |         |         |    |              |
| Missing                        | 1  | 1   |         |         |    |              |
| **Butting other**              |    |     |         | 0.31    |    |              |
| No                             | 124| 47  |         | 1.72    |    | (0.57-4.83)  |
| Yes                            | 11 | 9   |         |         |    |              |
| Average frequency of behaviour/calf (min – max) | 0.42 | 0.83 |         | 1.28 | | (1.00-1.67) |
| **Butted by other**            |    |     |         | 0.29    |    |              |
| No                             | 125| 48  |         | 0.37    |    | (0.63-3.32)  |
| Yes                            | 11 | 9   |         |         |    |              |
| Average frequency of behaviour/calf (min – max) | 0.1 | 0.2 |         | 1.45 | | (0.63-3.32) |
| Behaviour            | Yes          | No          | Average frequency of behaviour/calf (min – max) |
|----------------------|--------------|-------------|-----------------------------------------------|
| Cross-sucking other  | Yes          | 10          | 0.10 (0.58-5.14)                              |
|                      |              | 8           | 0.20 (1.52)                                  |
|                      |              |             | 1.78 (0.70-3.25)                             |
|                      |              |             | 0.27                                          |
|                      | No           | 120         | 0.42 (0.09-1.48)                             |
|                      |              | 53          | 0.16 (0.85)                                  |
|                      |              |             | 0.85 (0.50-1.23)                             |
|                      |              |             | 0.45                                          |
| Cross-sucked by other| Yes          | 9           | 0.17 (0.43-5.38)                             |
|                      |              | 5           | 0.16 (0.97)                                  |
|                      |              |             | 0.97 (0.60-1.40)                             |
|                      |              |             | 0.87                                          |
|                      | No           | 126         | 0.08 (0.34-3.93)                             |
|                      |              | 51          | 0.29 (1.57)                                  |
|                      |              |             | 1.22 (0.94-3.25)                             |
|                      |              |             | 0.13                                          |
| Displacing           | Yes          | 10          | 0.09 (0.21-2.86)                             |
|                      |              | 5           | 0.16 (1.32)                                  |
|                      |              |             | 1.32 (0.66-2.85)                             |
|                      |              |             | 0.42                                          |
|                      | No           | 124         | 0.06 (0.02-1.48)                             |
|                      |              | 52          | 0.02 (0.30)                                  |
|                      |              |             | 0.23                                          |
| Fighting             | Yes          | 7           | 0.05 (0.46-2.40)                             |
|                      |              | 1           | 0.09 (1.06)                                  |
|                      |              |             | 1.06 (0.46-2.40)                             |
|                      |              |             | 0.87                                          |
|                      | No           | 128         | 0.05 (0.01-1.80)                             |
|                      |              | 55          | 0.02 (0.30)                                  |
|                      |              |             | 0.23                                          |
| Headshake            | Yes          | 5           | 0.05 (0.46-2.40)                             |
|                      |              | 1           | 0.09 (1.06)                                  |
|                      |              |             | 1.06 (0.46-2.40)                             |
|                      |              |             | 0.87                                          |
|                      | No           | 130         | 0.05 (0.01-2.19)                             |
|                      |              | 55          | 0.09 (1.06)                                  |
|                      |              |             | 1.06 (0.46-2.40)                             |
| Licking other        | Yes          | 23          | 0.47 (0.72-3.83)                             |
|                      |              | 14          | 0.30 (0.86)                                  |
|                      |              |             | 0.86 (0.58-1.17)                             |
|                      |              |             | 0.39                                          |
|                      | No           | 112         | 0.47 (0.55-2.57)                             |
|                      |              | 42          | 0.30 (1.03)                                  |
|                      |              |             | 1.03 (0.70-1.48)                             |
| Licked by other      | Yes          | 35          | 0.47 (0.70-1.48)                             |
|                      |              | 16          | 0.46 (0.86)                                  |
|                      |              |             | 0.86                                          |
| Behaviour                        | Yes | No   | Average frequency of behaviour/calf (min – max) | Estimation not possible |
|----------------------------------|-----|------|-----------------------------------------------|------------------------|
| Locomotor play                   | Yes | 10   | 0.10 (0-2)                                    | 0.18 (0-4)             |
| Mounting                         | Yes | 5    | 0.04 (0-1)                                    | 0 (0-0)                |
| Mounted by other                 | Yes | 7    | 0.05 (0-1)                                    | 0 (0-0)                |
| Oral manipulation pen            | Yes | 36   | 0.55 (0-6)                                    | 0.77 (0-6)             |
| Self-rubbing                     | Yes | 38   | 0.44 (0-7)                                    | 0.45 (0-4)             |
| Rubbed by other                  | Yes | 15   | 0.13 (0-2)                                    | 0.11 (0-1)             |
| Rubbing on other                 | Yes | 12   | 0.10 (0-2)                                    | 0.04 (0-1)             |
| Self-licking                     | Yes | 85   | 1.94 (0-11)                                   | 2.14 (0-8)             |
| Stretching                       | Yes | 5    | 0.04 (0-1)                                    | 0.02 (0-1)             |
| Tongue playing/rolling | No  | 127 | 49 | 0.11 |
|------------------------|-----|-----|----|------|
| Yes                    | 8   | 7   |    | 2.65 (0.79-9.07) |
| Average frequency of behaviour/calf | 0.10 | 0.20 | 1.50 (0.78-2.87) | 0.21 |
Supplementary figure S3. Variable contributions to Principal Components associated with colonisation of VTEC O157:H7 in the Principal component regression model.
Supplementary Table S4. Description and result from sampling of pens indicated as positive for VTEC O157:H7 by environmental sampling.

| Farm | Date of sampling | Pen | Animals in pen | Sampled animals | Colonised animals | Shedding animals | Pen size (m²) | Bedding | Average age (days) | Comments |
|------|------------------|-----|----------------|-----------------|------------------|-----------------|--------------|---------|-------------------|----------|
| F1   | 2015-10-26       | 1   | 5              | 4               | 4                | 1               | 11           | Straw   | 94                |          |
|      |                  | 2   | 8              | 5               | 2                | 0               | 11           | Straw   | 106               |          |
|      |                  | 3   | 6              | 6               | 0                | 0               | 6            | Slatted floor/rubber | 149     |
|      |                  | 4   | 6              | 6               | 0                | 0               | 6            | Slatted floor/rubber | 227     |
|      |                  | 5   | 6              | 3               | 0                | 0               | 13           | Straw   | 127               | Sampling stopped after 3 animals due to stress in the group |
| F2   | 2015-11-16       | 1   | 11             | 8               | 1                | 0               | 32           | Straw   | 145               |          |
|      |                  | 2   | 12             | 5               | 1                | 1               | 36           | Straw   | 196               |          |
|      |                  | 3   | 10             | 6               | 0                | 0               | 29           | Straw   | 75                |          |
|      |                  | 4   | 11             | 7               | 0                | 0               | 30           | Straw   | 111               |          |
|      | 2015-10-20       | 1   | 20             | 10              | 1                | 1               | 59           | Straw   | 85                |          |
|      |                  | 2   | 10             | 1               | 0                | 0               | 79           | Straw   | 155               | Sampling stopped because of risk of injury |
|      |                  | 3   | 9              | 4               | 0                | 0               | 34           | Straw   | 101               |          |
|      |                  | 4   | 16             | 5               | 0                | 0               | 78           | Straw   | 148               |          |
| F4   | 2015-11-24       | 1   | 7              | 7               | 2                | 1               | 27           | Straw   | 111               |          |
|      |                  | 2   | 13             | 13              | 0                | 0               | 84           | Straw   | 158               |          |
| F5   | 2015-11-17       | 1   | 14             | 9               | 3                | 1               | 65           | Straw   | 154               |          |
|      |                  | 2   | 14             | 9               | 0                | 0               | 65           | Straw   | 137               |          |
|      |                  | 3   | 7              | 5               | 0                | 0               | 31           | Straw   | 97                |          |
|      |                  | 4   | 8              | 5               | 0                | 0               | 31           | Straw   | 82                |          |
| F6   | 2016-11-02       | 1   | 4              | 3               | 1                | 1               | 6            | Straw   | 51                |          |
|      |                  | 2   | 20             | 14              | 10               | 0               | 48           | Straw   | 99                |          |
|      |                  | 3   | 4              | 3               | 0                | 0               | 6            | Straw   | 50                |          |
|      |                  | 4   | 4              | 3               | 0                | 0               | 6            | Straw   | 33                |          |
|      |                  | 5   | 4              | 3               | 0                | 0               | 6            | Straw   | 34                |          |
| F7-1 | 2015-11-18       | 1   | 6              | 6               | 2                | 0               | 13           | Straw   | 49                |          |
|      |                  | 2   | 4              | 4               | 3                | 1               | 13           | Straw   | 95                |          |
|      |                  | 3   | 6              | 4               | 0                | 0               | 17           | Straw   | 12                | Two calves that were <48 hours old were not sampled |
|      |                  | 4   | 17             | 3               | 0                | 0               | 60           | Straw   | 127               | Sampling stopped after 3 animals because risk of injuries (No adequate possibilities for restraining) |
| F7-2 | 2016-11-03       | 1   | 7              | 7               | 3                | 1               | 13           | Straw   | 78                |          |
|       |       |       |       |       |       |       |       | Stock |   |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|---|
| 2     | 7     | 7     | 1     | 1     | 13    | Straw | 121   |       |   |
| 3     | 1     | 1     | 0     | 0     | 13    | Straw | 42    |       |   |
| 5     | 5     | 5     | 0     | 0     | 17    | Straw | 30    |       |   |
| **F8** | **2016-04-28** | 1     | 18    | 8     | 1     | 150   | Straw | 219   |   |
| 2     | 8     | 5     | 1     | 0     | 26    | Straw | 175   |       |   |
| 3     | 12    | 8     | 1     | 0     | 36    | Straw | 118   |       |   |
| 4     | 7     | 4     | 0     | 0     | 21    | Straw | 46    |       |   |
| **F9-1** | **2015-10-27** | 1     | 36    | 14    | 1     | 190   | Slatted floor/rubber | 176 |   |
| 2     | 8     | 5     | 2     | 2     | 20    | Straw | 98    |       |   |
| 3     | 10    | 5     | 2     | 1     | 22    | Straw | 101   |       |   |
| 4     | 11    | 5     | 0     | 0     | 22    | Straw | 76    |       |   |
| **F9-2** | **2016-06-03** | 5     | 8     | 7     | 4     | 2     | 22    | Straw | 101 |   |
| 2     | 6     | 6     | 0     | 0     | 20    | Straw | 121   |       |   |
| 3     | 7     | 7     | 0     | 0     | 22    | Straw | 139   |       |   |
| **F10** | **2016-06-28** | 1     | 12    | 12    | 2     | 2     | 25    | Straw | 61  |   |
| 2     | 13    | 7     | 1     | 0     | 128   | Straw | 195   | Poor restraining possibilities led to time running out |   |
| 3     | 6     | 6     | 0     | 0     | 30    | Sawdust | 134 |       |   |
| **F11** | **2016-04-06** | 1     | 4     | 4     | 3     | 0     | 4     | Straw | 71  |   |
| 2     | 6     | 6     | 2     | 0     | 8     | Slatted floor/rubber | 171 |       |   |
| 3     | 3     | 3     | 1     | 0     | 3     | Straw | 43    |       |   |
| 4     | 3     | 2     | 0     | 0     | 6     | Straw | 143   |       |   |
| 6     | 3     | 3     | 0     | 0     | 5     | Straw | 135   |       |   |
| 7     | 2     | 2     | 0     | 0     | 2     | Straw | 12    |       |   |
| **F12** | **2017-04-17** | 1     | 19    | 18    | 3     | 2     | 137   | Straw | 216 | Unsampled animal an older cow |

F8 Poor restraining possibilities led to time running out.