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Farm practices to control *E. coli* O157 in young cattle - A randomised controlled trial

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Abstract – A randomised controlled trial was used to investigate the effect of three complex management intervention packages to reduce the burden of *E. coli* O157 in groups of young-stock on cattle farms in England and Wales. All intervention farms were assigned measures to avoid buying in new animals and having direct contact or sharing water sources with other cattle. Furthermore, package A (7 farms) aimed to keep a clean environment and closed groups of young-stock; package B (14 farms) aimed for improved water and feed hygiene, whilst package C was assigned both A and B. The control farms (26 farms) were asked not to alter their practices. Farms, which were assigned intervention package A, exhibited a 48% reduction in *E. coli* O157 burden over the 4.5 months (average) of observation, compared to 18% on the control farms. The effect of package A compared to the control farms in a crude intention-to-treat model was RR = 0.26 ($p = 0.122$). When the risk ratio was adjusted for actual application of the different measures, the effect of intervention package A became stronger and statistically significant (RR = 0.14 $p = 0.032$). Statistical evidence ($p < 0.05$) showed that dry bedding and maintaining animals in the same groups were the most important measures within the package and weak evidence ($p < 0.1$) showed that a closed herd policy and no contact with other cattle may also be of importance. Compliance with the other measures in package A had no influence on the effect of the package. No evidence of effect of the other two intervention packages was found.

VTEC (STEC) *E. coli* O157 / randomised controlled trial / prevention / zoonotic control in cattle

1. INTRODUCTION

Verocytotoxigenic *Escherichia coli* O157 (*E. coli* O157) is a zoonotic pathogen of importance causing both outbreaks and sporadic human cases in England and Wales each year, where the number of confirmed cases increased by 36% from 2004 (699) to 2005 (950)[1]. Cattle have been identified as the main reservoir of *E. coli* O157 infections for humans and the traditional route of transmission from cattle to man is via contaminated meat. However, the proportion of infections acquired by direct contact with cattle or from contaminated environments such as fields or water courses is increasing [6].

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[1] Health Protection Agency, *E. coli* epidemiological data [on line] (2007) http://www.hpa.org.uk/infections/topics_az/ecoli/ O157/data_ew.htm [consulted 15 June 2007].

Cattle are asymptomatic carriers and the infection causes no production loss to the farmer. Young cattle between 2–18 months of age are at highest risk of excreting *E. coli* O157 through faeces or saliva [13]. Shedding by the individual animal is intermittent, probably due to re-circulation of the pathogens between animals or the environment and recent simulation models have suggested that the majority of transmission occurs through the environment, especially when young-stock are housed [10]. The load of bacteria shed by different animals may vary and it has been suggested that a few animals play an important role in transmission within a group of cattle [9, 19].

Today, control of *E. coli* O157 in England and Wales is focused on limiting transmission from cattle to humans. The Clean Animal Policy, which was introduced in 1996, prevents visibly dirty animals from being slaughtered and aims to reduce the risk of *E. coli* O157 along with other zoonotic pathogens contaminating meat[2]. Since 2000, between 1–7 commercial cattle farms have been traced annually in England and Wales as potential sources of human outbreaks and investigated by the authorities. Several farm-based studies have been conducted to identify risk factors, which can be targeted by on-farm control measures. Dirty water troughs, presence of other animal species, different feeds and various housing facilities have been reported to effect the risk, but few reports identify similar risk factors and consistent reports are rare [4, 11, 15, 17, 22].

The ubiquitous nature of *E. coli* O157 complicates identification of probable control points without structured studies to estimate the actual effect of control measures applied in the cattle population of interest. This paper presents the results of a randomised control trial (RCT) that was conducted in young cattle in England and Wales. The RCT examined the effect of farm practice changes on reduction of *E. coli* O157 burden in groups of young cattle. The objectives were (1) to establish the effect of three control packages of on-farm management practices to reduce *E. coli* O157 in young-stock; (2) to assess the effect of observed compliance with intervention measures on the result and (3) to establish the relative impact of various control measures within potentially effective control packages.

2. MATERIALS AND METHODS

2.1. Study design

Study farms were initially identified through private veterinary practices (PVP), which had submitted any kind of cattle samples for diagnosis to the Veterinary Laboratories Agency's regional laboratories (VLA RL) during the previous 12 months as previously described [4]. The cattle farms within each PVP, who submitted the largest number of samples in the previous year, were included and further suggestions of potentially interested farmers from the PVP were also accepted, however neighbouring farms were excluded. A total of 411 farms distributed throughout England and Wales were contacted by phone to assess willingness to participate in the study and eligibility of the herd by questionnaire. Farms were eligible, if they retained more than 60 cattle including 20 young-stock, had a bovine tuberculosis-negative status and the premises were not shared with any public access enterprises such as open farms, Bed & Breakfast or farm-shops including selling unpasteurised milk. A total of 156 farms were excluded due to ineligibility.

Sample sizes were calculated by a multilevel approach with design-effects and intra-class correlations deducted from variance between pats, groups and farms observed in a previous field study on a similar population [13]. The required samples sizes were 48 control farms and 48 farms in each intervention group to detect a risk ratio of 5 at 80% power with 95% confidence, when using a design effect of 13.2^2 to adjust for a group cluster size of 20 pat samples per group per visit. The design effect was estimated from data originating from a longitudinal study using same sampling approach along side individual animal sampling[3].

The remaining 255 farms were visited once in the second half of 2003 and twenty fresh floor faecal samples were collected from weaned young-stock (3–18 months of age) at each farm [4]. The

[2] Food Standards Agency, Clean livestock [on line] (2007) http://www.food.gov.uk/foodindustry/farmingfood/cleancattleandmeatsafety/ [consulted 4 January 2007].

[3] DEFRA, [on line] (2007) http://www2.defra.gov.uk/research/project_data/projects.asp?M=KWS&V=oz0138&SCOPE=1 [consulted 7 January 2007].

Table I. Allocation of farms and description of intervention packages used in a randomised controlled trial to reduce *E. coli* O157 in young-stock.

Interventions allocated	Package A	Package B	Package C	Control Package
None				√
No new animals brought in	√	√	√	
No contact with other cattle	√	√	√	
No shared water sources	√	√	√	
Keep bedding dry	√		√	
Keep animals clean	√		√	
Maintain closed group	√		√	
Use boot-dip	√		√	
Use overcoat	√		√	
Clean water troughs weekly		√	√	
Empty water troughs weekly		√	√	

farmer was asked to complete questionnaires regarding management details such as size and type of enterprise, cattle purchase, quarantine procedures, grazing, housing facilities, water sources and general hygiene procedures. If one or more samples were positive for *E. coli* O157, the farm was considered eligible for enrolment in the intervention study. A total of 23 of the 82 *E. coli* O157 positive farms were unwilling to participate in the trial, one farm was found to be bovine tuberculosis positive and one farm had sold its livestock. The remaining 57 farms were randomly allocated into three intervention groups and one control group (Tab. I). The allocation was done blindly by a clerk, who assigned each participating farm a random letter drawn from an envelope, which contained one letter for each intervention group and four for the control group.

Seven farms were allocated to intervention group A, 14 to intervention group B, 6 to intervention group C and 30 farms were allocated to the control group (Tab. I). A group of at least 20 weaned calves (3–18 months of age) was selected by convenience at each farm and the group-level interventions were applied to this monitored group of animals only. Only one group was monitored per farm and thus, group and farm are synonymous terms in this paper.

The effect of each intervention package on the burden of *E. coli* O157 in the group was compared to the burden in the control group, which was required to continue normal farming practices. Each intervention package contained several intervention measures, some of which were to be applied at farm level to all intervention farms and some measures, which were to be applied to a group of > 20 youngstock of 3–18 months of age on each farm (Tab. I).

2.2. Follow-up procedures

Four follow-up visits were conducted on each farm every 4 to 6 weeks by a member of staff from the VLA RL and the farms were monitored for 141 days (4.5 months) on average. The visits took place between October 2003 and May 2004, with 90% of all visits between November 2003 and April 2004. The staff member scored cleanliness of 5 animals, 1 in each corner and 1 in the middle of the enclosure as described by Ward et al. 2002 and used for the Food Standards Agency's Clean Animal Policy scores [23]. The condition of the bedding was assessed using a "squelch-score". A standardised method was used to score each corner and the middle of the enclosure from 1 (very dry) and 5 (soaked and slippery). The 5 squelch-scores and the 5 animals' cleanliness-scores were summarized separately to enclosure medians per visit, which were used in the data analyses. The variable was then dichotomised using a cut-off between 2 (= dry) and 3 (= squelchy sound). Training was provided to all staff to ensure standardisation within the scoring systems. In addition at each visit, standardised forms were used to record application of all intervention measures for each farm independently of the assigned intervention package. The farmers were compensated for their assistance.

Four farms allocated to the control group were lost during the follow-up period, 1 farm after 2 visits and 3 farms after 3 visits. All 4 farms were excluded from the analyses.

2.3. Faecal samples

Twenty samples were collected from freshly voided pats on the floor in the enclosure of the monitored group using a standardised "point of a compass"- approach to choosing pats in the enclosure (details are available on request). Each pat sample was analysed for the presence of *E. coli* O157 by immuno-magnetic separation (IMS) and suspected colonies were confirmed by latex slide agglutination as previously described [13]. Each sample was identified as positive or negative on standardised forms and entered into a Microsoft Access database by a trained clerk.

2.4. Application of interventions

Variables were created to describe the level of application of each measure throughout the study. Application was assessed for single measures within the packages and not the packages themselves. The application of each measure was assessed equally for all farms irrespective of nominal intervention package and registered as a binary variable presenting each intervention measure. A cut-off value of 2 was chosen for animal cleanliness scores and the squelch score to concur with the values used by the Food Standards Agency. Farms, which applied a specific measure 75% or more of the monitored time, were classified as applying the measure.

2.5. Data Analyses

2.5.1. Baseline measures

The success of randomisation was assessed by comparative univariable analyses of the baseline characteristics of herd sizes, percentage of enterprises, age interval in monitored groups and group sizes in the four allocated groups. Herd size was categorised in four categories using percentiles to accommodate changes over time and to allow for non-normality. Age of the group of animals was also categorised in four levels to reflect the within group variation of the age of the individual animals. The Kruskal–Wallis test was applied for tests on continuous variables, Pearson's X^2 for binary variables and k-sample test on ordinal variables [7].

2.5.2. Descriptive analyses

The application variables were described and compared for collinearity between each other and for collinearity with intervention group. Collinearity between cleaning and disinfecting pens before introducing animals and intervention group was found and the "cleaning and disinfecting pens"-variable was excluded from any multivariable analyses.

The degree of randomness in application was assessed by identifying potential associations between the applied measures and nominal allocated measures using Pearson X^2-tests. Application of several of the intervention measures were associated with nominal allocation status and application of a measure were considered non-random and non-ignorable, highlighting the need for analyses beyond intention-to-treat [2, 5].

The measured outcome was the number of *E. coli* O157 positive samples out of the 20 collected samples at each farm per visit. The proportion of positives was interpreted as burden of infection within the group of animals. The data were assessed visually for trends before choosing analytical approaches. The change in the proportion of positives samples that were detected at visit 1 and visit 4 was calculated for each group and the reduction attributable (reduction difference) to each intervention package was estimated by subtracting the change within the control group from that observed in each intervention group.

2.5.3. Measure of effect

The measure of effect was the risk ratio of positive samples between each intervention group and the control group after 4 months of participation in the study. The intervention package was considered effective if the risk ratio was less than 1 and statistically significant ($p < 0.05$). In order to evaluate the measure of effect, univariable as treated analysis (AT) and intention to treat (IT) was used. Ideally randomised trials should be evaluated with an IT model, however AT analysis was used in order to evaluate the measure independent of group allocation and adjust for the non-randomness of application [5].

2.5.4. Univariable as-treated analyses

AT analyses ignores the randomised allocation and "re-group" the farms according to actual application of intervention measures, whether or not the measure was allocated [2, 5]. Intuitively, this approach provides a more precise estimate of the effect of the measures, without the dilution of non-compliance. However, the analysis is biased, when the farmers, who apply, are not comparable to farmers, who not apply the measure. AT-analyses do no longer have the power of the randomisation, because adjustment for confounders and other differences between the groups should be included in the analyses, before effect can be assessed [5, 20].

The effect of each intervention measure actually applied on *E. coli* O157 burden was assessed by univariable comparative analysis [5]. A population-averaged approach based on generalised estimating equations (GEE) models to account for repeated measurements was applied. An autoregressive correlation matrix was assumed to represent the time-dependant correlation between effect and application of control measure using "visit" as the time-indicator. "Farm/group ID" was specified as a subject for the repeated measurement and the distributional family was defined as binomial specifying number of sampled faecal pats per visit. A logit function was used as the identity link and a robust variance estimator was included to provide valid standard errors, because the exact correlation structure of the data was unverified. An interaction term between the application variable and "visit" was included in each model to allow for changes in reduction over time and allow the measures of effect (risk ratios) to be calculated and compared at visit four, which was the last follow-up visit. A co-variable was included in each model to adjust for differences in the initial number of positive samples, but no other differences in baseline measures were adjusted for.

2.5.5. *Intention-to-treat analyses*

Intention-to-treat (ITT) analyses compares the effect of allocation independently of compliance and often provide a conservative estimate of effect "diluted" by non-compliance [2, 5]. The approach is based on the underlying assumptions that (1) compliance is random and (2) compliance patterns under the trial conditions mimic compliance patterns in society after trial completion. Bias is introduced because compliance is rarely random and behaviour is likely to change after trial completion due to removal of financial incentives or veterinary services, which are often provided to ensure participation.

The intention-to-treat effect of each intervention package on the proportion of *E. coli* O157 positive samples in young stock was analysed using a GEE model with similar specifications as described above for the AT analyses. "Intervention group" was used as explanatory variable and included as a fixed effect in the model. An interaction term between "intervention group" and "visit" was also included to allow for changes in effect between visits. The control group was coded with the lowest number in the "intervention group" variable and used as the baseline group, to which the three interventions groups were individually compared.

The ITT model was expanded into a multivariable model to evaluate the impact of compliance on the strength of the effect of intervention packages by including all the application variables in the model for adjustment of effect.

Impact of the single control measures included in package A, which showed weak evidence of effect, was investigated further by backward stepwise exclusion of the AT-variables in package A, where the least significant (Wald's test) co-variable was removed and the model rerun until all remaining variables in the model were significant. After every rerun the RR of the package was examined to ensure that no significant confounding AT variables were removed from the model. All significant measures in the final model were defined as important in the reduction of *E. coli* O157 and non-significant measures as less important. An additional group of control measures that showed weak evidence of association with the effect (p-values between 0.05–0.01) were identified as potentially important.

GEE-models are not likelihood-based models and methods to assess goodness-of-fit and adequacy are still under development and we were not able to assess the fit of our final model [1, 12].

All analyses were carried out in STATA 9 (Stata Corporation, College Station, TX, USA) and $p \leqslant 0.05$ was used as significance level in all analyses.

3. RESULTS

3.1. Baseline measures

Comparative analyses of baseline characteristics of the four allocated groups revealed no statistically significant differences and the randomisation was considered successful (Tab. II).

3.2. Descriptive analyses

The change in proportion of *E. coli* O157 positive samples was an overall decline in all four groups over the four and a half trial months (Tab. III). The average reduction was 0.48 in group A, 0.19 in group B, 0.32 in group C and 0.18 in the control group (Tab. III). A total reduction of 0.30 could be attributed to intervention group A, 0.01 to intervention

Table II. Baseline characteristics of three intervention groups and a control group in a randomised controlled trial.

	Variable type	Control	A	B	C	*p*-values for heterogeneity
Number of farms		26	7	14	6	N/A
Size of cattle enterprise (median[a])	Categorical	180–300	301–450	180–300	301–450	0.130[b]
Presence of dairy enterprise (%)	Continuous	81	100	79	100	0.556[c]
Presence of beef enterprise (%)	Continuous	50	29	36	17	0.444[c]
Age-interval[d] in groups (median)	Categorical	6–12	6–12	12–18	6–12	0.239[e]
Number of animals in group	Continuous	45	28	37	29	0.223[c]

[a] Categories of herd sizes; [b] k-test for differences in medians; [c] X^2-test; [d] Categories of age-intervals (youngest-oldest) in groups (months); [e] Kruskal-Wallis test.

Table III. Proportion of *E. coli* O157 positive samples and (standard deviations) in intervention groups by visit during a four and half months trial.

Intervention groups	Visits			
	1	2	3	4
Control	0.35 (0.34)	0.19 (0.27)	0.23 (0.24)	0.15 (0.24)
A	0.56 (0.45)	0.31 (0.37)	0.25 (0.29)	0.08 (0.19)
B	0.40 (0.37)	0.25 (0.32)	0.26 (0.31)	0.21 (0.34)
C	0.53 (0.43)	0.20 (0.36)	0.55 (0.33)	0.21 (0.32)

Table IV. Percentage of farms applying control measures by nominal allocation status in a RCT and comparison of these proportions to assess randomness of application.

Intervention measure	Allocated (%)	Not allocated (%)[a]	*p*-value[b]
No water shared with other cattle	77.8	62.5	0.012
No direct contact with other cattle	81.5	76.9	0.433
No new animals bought into herd	88.9	92.3	0.382
Keep bedding dry	55.8	72.5	0.024
Clean pens before animals housed	0.0	15.0	0.003
Keep animals clean	82.5	76.9	0.372
Use of boot-dip	78.4	2.5	< 0.001
Use of overcoat	39.2	2.5	< 0.001
Keep animals in same groups	92.3	32.5	< 0.001
Empty water troughs weekly	60.0	6.5	< 0.001
Clean water troughs weekly	55.5	60.6	0.688
Raise water troughs to animal chin height	17.7	12.9	0.350

[a] Including control group; [b] X^2-test for significance.

group B and a 0.14 reduction to intervention group C, when compared to the control group.

3.3. Compliance

Compliance with allocated intervention measures throughout the study was inconsistent and none of the farms complied 100% with all measures included in the package. The measure, most frequently complied with was "not buying in new animals" (85.7% in A, 92.9% in B and 82.6% in C). The measure least frequently complied with was "raising water troughs to animal chin height", which was only applied in 23.1% of B farms and 0.0% of C farms. Application of measures was similar in groups, to which they were allocated, apart from "avoiding sharing water sources", which was done more consistently in group A than in group C (p = 0.009) and

Table V. Crude risk ratios comparing the effect on *E. coli* O157, when applying a measure for four months compared to not applying the measure.

Intervention measure	Applied (Farms)	Not applied (Farms)	Control of VTEC O157 burden (Crude RR[a])	*p*-value (Walds[a])
No water shared with other cattle	36	15	1.1	0.795
No direct contact with other cattle	11	42	2.4	0.046
No new animals bought into herd	48	5	1.2	0.714
Keep bedding dry	37	16	1.2	0.387
Clean pens before animals housed	6	47	1.9	0.171
Keep animals clean	43	10	0.6	0.449
Use of boot-dip	11	42	0.7	0.577
Use of over-coat	6	47	0.7	0.547
Keep animals in same groups	25	28	1.3	0.823
Empty water troughs weekly	14	37	1.3	0.425
Clean water troughs weekly	31	22	1.6	0.224
Raise water troughs to animal chin height	7	41	1.5	0.533

[a] Obtained using GEE modelling, univariable results, no adjustment for confounding factors.

"raising the water troughs to chin height", which was applied more often in B than C ($p = 0.04$).

Five intervention measures were applied with equal frequency in all groups independently of allocation (Tab. IV). Application of the seven remaining measures was associated with nominal allocation, which implied that compliance was not a totally random process and should not be ignored, when interpreting the effects of the intervention packages.

3.4. Univariable as-treated analysis

The number of farms, which applied the individual intervention measures, is shown in Table V. The classification of application status was done independently of allocation and only one of the individual measures was directly associated with *E. coli* O157 positive samples on farms in the crude univariable comparisons (Tab. V). No direct contact with other cattle increased the risk of *E. coli* positive samples. However, the analyses were not adjusted for confounders or differences between the farms that did and did not apply the measures and the exact value of RR should be interpreted with caution.

3.5. Intention-to-treat analysis

Visually, intervention package A reduced the number *E. coli* O157 positive samples within a group of young-stock over a four and a half months period more effectively than the control group albeit this reduction was not statistically significant (RR = 0.26; CI_{95}:0.05-1.43, $p = 0.122$) (Fig. 1). The RCT provided no evidence of an effect of intervention packages B (RR = 1.37, $p = 0.631$) or intervention package C (RR = 1.27, $p = 0.671$) on *E. coli* O157, when compared to the control group.

The model was extended to a multivariable model, where all application variables were included to adjust for compliance. This model revealed that a significantly greater rate of reduction of *E. coli* O157 was observed in intervention group A than in the control group (Tab. VI). No evidence of a significant effect of intervention group B and C was found.

The significant reduction in *E. coli* O157 observed in intervention group A (RR 0.14; $p = 0.032$) was a result of applying several control measures. The effect (RR) remained stable and significant throughout most of the stepwise removal of application variables until "dry bedding" and "keep animals in same groups" were removed at which point the RR of the package increased considerably. This suggested that "keeping the bedding dry" and "keeping animals in the same groups" were the most important factors to apply to ensure effectiveness of the intervention package

Table VI. The effects of three intervention packages to reduce *E. coli* O157 over a four and a half months period, when adjusted for actual compliance of all intervention measures.

	Coefficient[a]	Risk ratio	95% CI	*p*-value[b]
Intervention A	–0.63	0.14	0.02–0.84	0.032
Intervention B	0.07	0.80	0.20–3.10	0.744
Intervention C	0.10	0.77	0.14–4.34	0.768

[a] Interaction term with visit; [b] Estimates from multivariate GEE model.

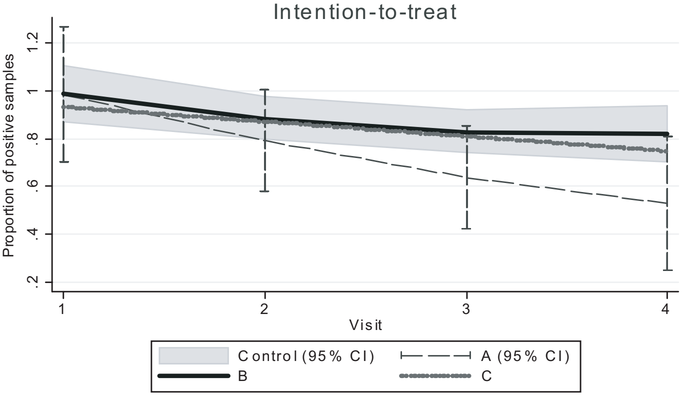

Figure 1. Reductions on the burden of *E. coli* O157 by three intervention packages and a control group after adjusting for difference in initial difference.

(Tab. VII). Other measures of slightly less importance were whether new animals were bought into the herd during the study period or whether the cattle had direct contact with animals from other farms (both $p < 0.1$). The remaining variables describing the measures in intervention package A showed no evidence of effect on reduction of the *E. coli* O157 burden. The non-significant were: “use of pen-specific overcoats and boot-dip”, “not share water sources with other cattle” and “keep the cattle clean”.

Table VII. Significance of compliance with various measures included in intervention package A on the reduction of *E. coli* O157 in multivariate GEE-model.

Intervention measure	*p*-value (Walds[a])
Keep animals in same groups[a]	0.01
Keep bedding dry[a]	0.01
No direct contact with other cattle	0.07
No new animals bought into herd	0.07
Use of over-coat	0.55
No water shared with other cattle	0.56
Keep animals clean	0.92
Use of boot-dip	0.99

[a] Included in final model.

4. DISCUSSION

The RCT revealed that it is possible to reduce the overall burden of *E. coli* O157 on farms at group-level by simple management changes. A combined intervention package, which was intended to avoid introduction or re-introduction of *E. coli* O157 into a group of young-stock by using designated boot-dips and over coats and keep animals in the same group along with keeping animals clean, applying farm-level biosecurity measures and maintaining dry bedding significantly reduced the level of *E. coli* O157 in a group of young-stock within a four and a half months period of application. We have also identified the measures, which provided the greatest contribution to the reduction in the intervention package. Keeping young cattle in the same groups throughout rearing without introducing new animals and ensuring constant dry bedding appeared to have a particularly large impact on the reduction. The model also provided us with weak evidence that not buying new stock into the herd and avoiding direct contact with cattle from other farms (i.e. shows, nose-to-nose contact over fences) provided some protection and may have played a role in the effect of the intervention package.

Young cattle reared together and similarly exposed with no introduction of new animals may develop similar immune-status to any *E. coli* O157 circulating between the animals and the environment. Introduction of an immunologically naïve animal or an animal carrying another type of *E. coli* O157 may upset the host-pathogen balance and provoke excessive shedding and increased burden of the pathogen by disrupting the previous group dynamics. Furthermore, introduction of a new animal into an established unit may induce stress in the group, which may also affect factors such as shedding rates or host-susceptibility. The same principles may account for the weak effect of bringing new animals into an established herd. *E. coli* O157 is shed in faeces, saliva and by direct contact, such as nose-to-nose contact with other cattle over fences or at shows, which may introduce or re-introduce the pathogen into a herd and trigger a disruption of status quo [21].

Intuitively, dry bedding was expected to be a predictor of the cleanliness of the animals. However, we found no association between the two measures and the application of keeping animals clean was not observed to be important for the effect of package A. Dryness of bedding probably did contribute to keeping animals clean, but dry and clean bedding may also have provided protection by other means [18]. Wet and dirty bedding can create a moist and warm environment for enhanced survival and potential growth of *E. coli*, which may have provided a constant reservoir and increased the burden of infection within the group [23]. Wet or dirty bedding has not been reported as a direct risk factor for *E. coli* O157 in other cattle populations, but less specific implications have been published. Several studies from Scotland have indicated an increased risk of shedding *E. coli* O157 if the animals were housed and this effect may be related to the bedding, which accounts for a large proportion of the near-environment of housed young-stock [22]. A similar non-specified association with access to straw was reported by Rugbjerg et al. [15].

A reduction in *E. coli* O157 burden within group C similar to that of group A was expected as group C was also asked to apply intervention package A, but this was not observed. Unfortunately, group C was too small to statistically explore all potential conflicting effects of measures in package A and B, but farmers did inform us that emptying the water-troughs weekly complicated keeping the bedding dry. This contradictory effect was further supported by a statistically significant association between emptying water-troughs and wet bedding (X^2: $p = 0.008$) and may impact on the effectiveness of package C especially since dry bedding had a high impact on *E. coli* O157 reduction in package A.

The effect of the interventions on individual animals was not considered in this study, because all interventions were applied at either group or farm-level. To avoid the atomistic fallacy of extrapolating individual results to group-level, all data collection and analyses were conducted at group-level. However, testing at group-level by using floor pats instead of rectal samples from individual animals may have resulted in an under-estimation of the proportion of positive samples in the groups. Floor samples are reported to have a 86% sensitivity compared to rectal samples from individual animals [21]. Nevertheless, the underestimation was considered non-differential and thus would not bias the comparative analyses. Supershedders or any other individual animals were not targeted in particular, with these interventions, because the aim was to recommend practical interventions that farmers can incorporate in their daily routine without additional costs such as veterinary bills or laboratory testing.

It was not possible to enrol as many farms as required according to pre-study sample size calculations. The consequent reduction in the power of the study may have precluded statistically significant associations between any of the individual measures or effects of intervention packages B and C and these results should be considered inconclusive. Nevertheless, the strong effect of intervention group A was identified as significant, despite the low statistical power, which suggested a very strong effect of this package.

RCTs are viewed as the ultimate study to establish causality or effect of preventive measures, because the pre-trial randomisation minimises the introduction of bias, which could reduce the comparability of the intervention and the control group [3]. In theory, the only difference between the two groups is the applied intervention measure(s), in reality, bias may be introduced both pre-randomisation and post-randomisation [14]. Pre-randomisation bias is usually non-differential selection bias, which can occur, if the groups do not represent the population intended. In our study, the farms were not chosen at random from the English and Welsh cattle population and non-representativeness was likely. However, at the initial screening stage of enrolment the herd-level prevalence was 32.2%, which was very similar to the national herd level prevalence of 38.9% [4, 13].

Very few RCTs in veterinary medicine have been published and to our knowledge none have been used to assess the effect of multiple sanitary control strategies in cattle herds. Sanitary measures and management practices are not isolated factors with specific, individual or random effects and even in publications in human medicine, where RCTs are more common, the combined effect of various measures and different levels of compliance and application is relatively unexplored. We applied methods used in publication in human medicine to obtain results, which are relevant to farming communities and policy-makers [2, 5, 16].

Each intervention package was complex and consisted of up to 26 measures (summarised in 12 variables in this paper) for the farmer to implement into his daily routines and no farmer complied with all of the measures in the allocated package all the time. It was not possible to assess AT efficacy of the intervention packages, so we chose to assess the efficacy of single measures instead. AT analyses identify the actual effect of applying a measure on the outcome, but because of potential confounding and incomparability between groups introduced in post-randomisation assignment of measures, the results were of limited relevance [5, 8]. None of the measures were associated with a reduction in *E. coli* O157 in the univariable analyses, which may be attributed to three main reasons: (1) Insufficient power to identify an association though present; (2) differences in the baseline characteristics of the AT groups that confounded and diluted a potential association and (3) none of the measures were individually efficient enough to induce a reduction in *E. coli* O157 and composite measures are necessary.

An ITT effect ignores any bias or confounding caused by non-compliance by returning a population-based efficacy, with the assumption that patterns of compliance will stay constant over time in the same population [8]. However, a proven effect of an intervention measure or introduction of a strong incentive i.e. payments or penalties, are likely to change compliance patterns in a population and thus, ITT proven efficacy may not be valid even in the same population in the future or after policy making. We have observed that the compliance pattern in our study group changed once the study was completed and incentives removed and we predict it may change again if an official control programme is implemented[4]. The predicted ITT effect by intervention package A may not represent the effect in the same population with a different compliance pattern as compliance was a strong confounder on the effect of the packages.

We failed to directly quantify the relative impact of each measure within the intervention packages, which was a sought-after outcome. However, we did identify four measures of strong or medium importance. Identification of a few, but efficient measures may facilitate

[4] Iversen J., Investigating the effect of motivating factors to implement a control programme using experiences from a RCT aiming to reduce VTEC O157 in cattle, Proc. ISVEE XI (2006).

policy decisions and increase the likelihood of a successful control programme. Adoption of a few intervention measures is more likely to be undertaken by the farming community than a large complex package of changes to their normal practices.

Implementation of a control programme for *E. coli* O157 on cattle farms has to overcome the major challenge of compliance to become successful, because reduction or elimination of *E. coli* O157 does not provide any economical benefits to the farming enterprise. To date, no structured on-farm control of *E. coli* O157 in commercial cattle farms exists in England and Wales and although this study provided evidence of simple measures that may be effective, incentives for farmers should be carefully considered before implementation of an official control programme.

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REFERENCES

[1] Albert P.S., Longitudinal data analysis (repeated measures) in clinical trials, Stat. Med. (1999) 18:1707–1732.

[2] Cuzick J., Edwards R., Segnan N., Adjusting for non-compliance and contamination in randomized clinical trials, Stat. Med. (1997) 16:1017–1029.

[3] Dohoo I., Martin W., Stryhn H., Veterinary Epidemiologic Research, 1st ed., AVC Inc., Charlottetown, 2003.

[4] Ellis-Iversen J., Smith R.P., Snow L.C., Watson E., Millar M.F., Pritchard G.C., Sayers A.R., Cook A.J.C., Evans S.J., Paiba G.A., Identification of management risk factors for VTEC O157 in youngstock in England and Wales, Prev. Vet. Med. (2007) doi:10.1016/j.prevetmed.2007.05.004.

[5] Greenland S., An introduction to instrumental variables for epidemiologists, Int. J. Epidemiol. (2000) 29:722–729.

[6] HPA, Vero cytotoxin-producing *Escherichia coli* O157:2005, Commun. Dis. Rep. CDR Wkly (2006) 16.

[7] Mann H.B., Whitney D.R., On a test whether one of two random variables is stochastically larger than the other, Annals of Mathematical Statistics (1947) 18:50–60.

[8] Mark S.D., Robins J.M., A method for analysis of randomized trials with compliance information: an application to the mulitple risk factor intervention tria, Control. Clin. Trials (1993) 14:79–97.

[9] Matthews L., Low J.C., Gally D.L., Pearce M.C., Mellor D.J., Heesterbeek J.A., Chase-Topping M., Naylor S.W., Shaw D.J., Reid S.W., Gunn G.J., Woolhouse M.E., Heterogeneous shedding of *Escherichia coli* O157 in cattle and its implications for control, Proc. Natl. Acad. Sci. USA (2006) 103:547–552.

[10] Matthews L., McKendrick I.J., Ternent H., Gunn G.J., Synge B., Woolhouse M.E., Super-shedding cattle and the transmission dynamics of *Escherichia coli* O157, Epidemiol. Infect. (2006) 134:131–142.

[11] Nielsen E.M., Tegtmeier C., Andersen H.J., Gronbaek C., Andersen J.S., Influence of age, sex and herd characteristics on the occurrence of Verocytotoxin-producing *Escherichia coli* O157 in Danish dairy farms, Vet. Microbiol. (2002) 88:245–257.

[12] Omar R.Z., Wright E.M., Turner R.M., Thompson S.G., Analysing reapeated measurements data: A practical comparisons of methods, Stat. Med. (1999) 18:1587–1603.

[13] Paiba G.A., Wilesmith J.W., Evans S.J., Pascoe S.J., Smith R.P., Kidd S.A., Ryan J.B., McLaren I.M., Chappell S.A., Willshaw G.A., Cheasty T., French N.P., Jones T.W., Buchanan H.F., Challoner D.J., Colloff A.D., Cranwell M.P., Daniel R.G., Davies I.H., Duff J.P., Hogg R.A., Kirby F.D., Millar M.F., Monies R.J., Nicholls M.J., Payne J.H., Prevalence of faecal excretion of verocytotoxigenic *Escherichia coli* O157 in cattle in England and Wales, Vet. Rec. (2003) 153:347–353.

[14] Peduzzi P., Henderson W., Hatigan P., Lavori P., Analysis of randomized controlled trials, Epidemiol. Rev. (2002) 24:26–38.

[15] Rugbjerg H., Nielsen E.M., Andersen J.S., Risk factors associated with faecal shedding of verocytotoxin-producing *Escherichia coli* O157 in eight known-infected Danish dairy herds, Prev. Vet. Med. (2003) 58:101–113.

[16] Sato T., A method for the analysis of repeated binary outcomes in randomised clinical trials with noncompliance, Stat. Med. (2001) 20:2761–2774.

[17] Schouten J.M., van de Giessen A.W., Frankena K., De Jong M.C., Graat E.A., *Escherichia coli* O157 prevalence in Dutch poultry, pig finishing and veal

herds and risk factors in Dutch veal herds, Prev. Vet. Med. (2005) 70:1–15.

[18] Scott G.B., Kelly M., Cattle cleanliness in different housing systems, Farm Building Progress (1989) 95:21–24.

[19] Shaw D.J., Jenkins C., Pearce M.C., Cheasty T., Gunn G.J., Dougan G., Smith H.R., Woolhouse M.E., Frankel G., Shedding patterns of verocytotoxin-producing *Escherichia coli* strains in a cohort of calves and their dams on a Scottish beef farm, Appl. Environ. Microbiol. (2004) 70:7456–7465.

[20] Small D.S., Ten Have T.R., Joffe M.M., Cheng J., Random effects logistic models for analysing efficacy of a longitudinal randomized treatment with non-adherence, Stat. Med. (2006) 25:1981–2007.

[21] Smith D.R., Gray J.T., Moxley R.A., A diagnostic strategy to determine the Shiga toxin-producing *Escherichia coli* O157 status of pens of feedlot, Epidemiol. Infect. (2004) 132:297–302.

[22] Synge B.A., Chase-Topping M.E., Hopkins G.F., McKendrick I.J., Thomson-Carter F., Gray D., Rusbridge S.M., Munro F.I., Foster G., Gunn G.J., Factors influencing the shedding of verocytotoxin-producing *Escherichia coli* O157 by beef suckler cows, Epidemiol. Infect. (2003) 130:301–312.

[23] Ward W.R., Hughes J.W., Faull W.B., Cripps P.J., Sutherland J.P., Sutherst J.E., Observational study of temperature, moisture, pH and bacteria in straw bedding, and faecal consistency, cleanliness and mastitis in cows in four dairy herds, Vet. Rec. (2002) 151:199–206.