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TITLE: Assessing the impact of tailored biosecurity advice on farmer behaviour and pathogen presence in beef herds in England and Wales

AUTHORS: **J.M. Cardwell**, S. Van Winden, W. Beauvais, A. Mastin, W.A. De Glanville, J. Hardstaff, R.E. Booth, J. Fishwick, D.U. Pfeiffer

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1 **ABSTRACT**

2 The term 'biosecurity' encompasses many measures farmers can take to reduce the risk of pathogen  
3 incursion or spread. As the best strategy will vary between settings, veterinarians play an important  
4 role in assessing risk and providing advice, but effectiveness requires farmer acceptance and  
5 implementation. The aim of this study was to assess the effectiveness of specifically-tailored  
6 biosecurity advice packages in reducing endemic pathogen presence on UK beef suckler farms. One  
7 hundred and sixteen farms recruited by 10 veterinary practices were followed for three years. Farms  
8 were randomly allocated to intervention (receiving specifically-tailored advice, with veterinarians and  
9 farmers collaborating to develop an improved biosecurity strategy) or control (receiving general  
10 advice) groups. A spreadsheet-based tool was used annually to attribute a score to each farm  
11 reflecting risk of entry or spread of bovine viral diarrhoea virus (BVDV), bovine herpesvirus-1 (BHV1),  
12 *Mycobacterium avium subsp. paratuberculosis* (MAP), *Leptospira interrogans* serovar *hardjo* (*L.*  
13 *hardjo*) and *Mycobacterium bovis* (*M.bovis*). Objectives of these analyses were to identify evidence of  
14 reduction in risk behaviours during the study, as well as evidence of reductions in pathogen presence,  
15 as indications of effectiveness. Risk behaviours and pathogen prevalences were examined across study  
16 years, and on intervention compared with control farms, using descriptive statistics and multilevel  
17 regression. There were significant reductions in risk scores for all five pathogens, regardless of  
18 intervention status, in every study year compared with the outset. Animals on intervention farms were  
19 significantly less likely than those on control farms to be seropositive for BVDV in years 2 and 3 and  
20 for *L.hardjo* in year 3 of the study. Variations by study year in animal-level odds of seropositivity to  
21 BHV1 or MAP were not associated with farm intervention status. All farms had significantly reduced  
22 odds of BHV1 seropositivity in year 2 than at the outset. Variations in farm-level MAP seropositivity  
23 were not associated with intervention status. There were increased odds of *M. bovis* on intervention  
24 farms compared with control farms at the end of the study. Results suggest a structured annual risk  
25 assessment process, conducted as a collaboration between veterinarian and farmer, is valuable in  
26 encouraging improved biosecurity practices. There were some indications, but not conclusive

27 evidence, that tailored biosecurity advice packages have potential to reduce pathogen presence.  
28 These findings will inform development of a collaborative approach to biosecurity between  
29 veterinarians and farmers, including adoption of cost-effective strategies effective across pathogens.

30

31 **Keywords:** biosecurity, beef cattle, tailored biosecurity advice, bovine viral diarrhoea, leptospirosis,  
32 bovine herpesvirus-1

33

34

## 35 Introduction

36 The farmer, as a decision-maker in relation to livestock management, is a key player in the control of  
37 livestock diseases. There are many measures a farmer can take to reduce the likelihood of a pathogen  
38 being introduced and spread on the farm, which are encompassed by the broad term 'biosecurity'.  
39 Measures likely to be effective against specific cattle pathogens have been identified by risk factor  
40 studies. For example, modifiable factors such as buying in new stock and use of communal grazing are  
41 associated with increased risk of introduction of bovine viral diarrhoea virus (BVDV) to herds (Presi et  
42 al., 2011). Dias et al. (2013) found that buying in cattle, use of a bull (natural service) and renting  
43 pasture from other farmers were risk factors for bovine herpesvirus infection. Buying in cattle and the  
44 presence of other ruminants on the farm have been identified as a risk factor for *Leptospira* spp.  
45 infection (Schoonman and Swai, 2010; Williams and Winden, 2014). There is strong evidence that  
46 buying in cattle is an important risk factor for the introduction of *Mycobacterium avium subsp.*  
47 *paratuberculosis*, while the impact of the presence of other ruminant species is less clear (Rangel et  
48 al., 2015). Buying in cattle, long-term storage of manure and use of silage clamps have been associated  
49 with TB breakdowns (Reilly and Courtenay, 2007). Some measures, such as maintaining a closed herd  
50 or reducing contact with other animals, are therefore likely to be effective against the introduction of  
51 more than one pathogen, where transmission characteristics are shared or similar (van Schaik et al.,  
52 1999; Cowie et al., 2014; Williams and Winden, 2014). However, identification of risk factors does not  
53 equate to demonstrating effectiveness of modifying these factors in real world contexts. One  
54 challenge is that any effective biosecurity risk management strategy will usually comprise multiple  
55 components, with each component possibly contributing a relatively small effect and the relative  
56 importance of different components varying between farms. Furthermore, in order for the strategy to  
57 be implemented it must be credible to farmers and feasible in their personal context. Even then, many  
58 other factors, such as personality, experience, education (Racicot et al., 2012), perceptions,  
59 knowledge and attitudes (Toma et al., 2013; Toma et al., 2015) all play a role in determining the likely  
60 uptake of advice by farmers. Advice is more likely to be followed if it is tailored to farmers' individual

61 contexts and characteristics rather than generic (Enticott et al., 2012; Jensen et al., 2016), and  
62 negotiated directly with them through a participatory approach (Enticott et al., 2012; Gosling et al.,  
63 2014; Duval et al., 2016) with veterinarians seen as valuable interpreters of generic advice (Garforth,  
64 2015). Farm veterinarians, because of their knowledge of pathogens and disease as well as of the  
65 specific characteristics and circumstances of individual farms and farmers, should therefore be ideally  
66 positioned to advise effectively on individually-tailored biosecurity strategies. While it has been  
67 reported that the preferred and most influential source of advice for many farmers is their own vet  
68 (Brennan and Christley, 2013; Jones et al., 2015), it has also been acknowledged that veterinary advice  
69 is not always followed even when perceived to be useful by farmers (Brennan and Christley, 2013).  
70 Therefore, the likely effectiveness of veterinary-led individually-tailored biosecurity strategies, in  
71 terms of reduction of pathogen presence or even uptake of advice, has not been established. This  
72 intervention study was designed to assess the effectiveness of biosecurity advice packages specifically  
73 tailored to individual beef suckler farms, provided by veterinary practitioners via a participatory  
74 approach, in changing farmer behaviour and in reducing the risk of introduction or spread of infectious  
75 diseases. Objectives of the analyses presented here were (i) to identify evidence of risk behaviours  
76 changing in response to tailored veterinary biosecurity advice packages; (ii) to identify evidence of  
77 reductions in farm-level and within-farm prevalence of five important bovine pathogens: bovine viral  
78 diarrhoea virus (BVDV), bovine herpes virus-1 (BHV1, the causative agent of infectious bovine  
79 rhinotracheitis [IBR]), *Leptospira interrogans* serovar *hardjo* (*L.hardjo*), *Mycobacterium avium*  
80 subspecies *paratuberculosis* (*MAP*, the causative agent of *Johne's disease*), and *M. bovis*  
81 (*Mycobacterium bovis*, the causative agent of bovine tuberculosis), in association with such advice.

82

## 83 **Materials and Methods**

84 A randomised controlled intervention study was conducted on beef suckler farms in South West  
85 England and Wales, an area where all five infectious diseases are prevalent. Veterinarians from ten  
86 collaborating veterinary practices (one veterinarian per practice) each recruited between 8 and 18

87 farms, with the larger practices recruiting proportionally more farms. Inclusion criteria were that  
88 farms should have at least 30 breeding females, at least 50 overall heads of stock, a beef suckler  
89 enterprise that was more than 50% of the farm business, as well as willingness to participate. Nested  
90 within practice, farms were randomly allocated to either the intervention group, which received  
91 detailed, specifically-tailored biosecurity advice packages, or the control group, which received only  
92 generic advice provided by the veterinarian as part of the normal consultation process. Sample size  
93 estimations indicated that a total of 120 herds would be sufficient to detect a risk ratio effect of 0.32  
94 with 80% statistical power, at a 5% significance level for a one-sided test assuming a cumulative  
95 disease incidence of 30% in the control group over the 2-year study period, and a ratio of 1:1 between  
96 controls (n=60) and intervention herds (n=60). The group size would still detect a risk ratio of 0.29 if  
97 10% of herds within each group were lost to follow-up. In total, 57 intervention and 59 control farms  
98 were recruited. Ethical approval for the study was obtained through the Royal Veterinary College's  
99 ethical review process. Data collection began in early 2008 and finished in mid-2012.

100

#### 101 *Biosecurity scoring tool development*

102 The ten participating veterinarians were briefed on previous work in which a farm-specific computer-  
103 based risk scoring tool for *M. bovis* had been developed (Van Winden et al., 2005; Van Winden and  
104 Aldridge, 2008). In order to create a tool capable of capturing the risks for the five specific pathogens  
105 under consideration in the current study, we adapted the evidence available at the time, which  
106 included generic risk factor categories such as cattle purchasing, direct and indirect contact with other  
107 cattle, ruminants and other animals, use of shared equipment and types of visitors to the farm, as  
108 reviewed by van Winden et al. (2005). These broad categories were divided into sub-factors to provide  
109 more detail. For example, cattle introductions were provided with details such as age, type of source  
110 (e.g. through a dealer, market, auction, etc.), number of sources and pathogen barriers provided  
111 (quarantine and testing). To elucidate risk factor weightings, the veterinarians took part in two expert  
112 opinion workshops. During the workshops, veterinarians were asked to allocate weights to reflect the

113 relative importance of specific sub-factors, such that the total weight of all sub-factors with each broad  
114 risk factor category would be 100%, in an approach similar to that used in the Competing Values  
115 Framework (Cameron and Quinn, 2006). In addition, veterinarians evaluated the importance of herd  
116 size for risk of pathogen introduction and spread. A semi-Delphi approach was used in order to achieve  
117 near-consensus (Gallagher, 2004; Banwell et al., 2005). After the first workshop the median of the  
118 proposed weights for each risk factor was presented and discussed in the second workshop. After the  
119 second workshop the veterinarians completed the process once again and the medians of these  
120 weights were subsequently used to construct the scoring tool in Microsoft Excel™, with creation of an  
121 algorithm to generate higher scores for farms with higher risk for disease introductions or spread and  
122 a lower scores for more biosecure units. Herd size weightings were used to create a curvilinear  
123 multiplier which generated increasing scores with increasing animal numbers. The final version of the  
124 scoring tool, including the median weightings for each risk factor, is presented in the supplemental  
125 material. The overall biosecurity score is the sum of factors contributing to the overall risk and the  
126 spreadsheet identifies the main risk contributor. This allowed farmers and veterinarians to identify  
127 specific factors that could be targeted for change during the following year, and by altering these  
128 factors an aspirational score could be generated. Before the scoring tool was used on the farms,  
129 training was provided for participating veterinarians, to familiarise them with the spreadsheet and to  
130 address any concerns.

131

### 132 *Data collection*

133 Risk measures:

134 Veterinarians visited all farms annually to complete the risk assessment questionnaire, recording  
135 existing biosecurity practices, resulting in 4 risk assessments per farm. In the analyses presented here,  
136 year 0 therefore represents the data collected at the start of the study and years 1-3 represent data  
137 collected at the end of year 1, 2 and 3 of the study. Initially, farm vaccination history was not recorded,  
138 but following discussions with participating veterinarians at the end of year 1, farm-level vaccination

139 status for BVDV, BHV1 and *L.hardjo*, reflecting relevant vaccinations in the previous year, were  
140 recorded thereafter.

141

142 Indicators of pathogen presence:

143 Blood samples for BVDV, BHV1, *L.hardjo* and MAP antibody testing were obtained from approximately  
144 fifty animals per farm and sent to the National Milk Records group laboratories for analysis. On each  
145 farm, 20 youngstock (9-21 months) and 30 adult cows (>2 years) were randomly selected each year  
146 for testing. The veterinarians were asked to sample the youngstock before their first vaccination, so  
147 they would act as sentinels. Random selection of adult cows meant that some individuals may have  
148 been sampled more than once, particularly in smaller herds. The sensitivities (Se) and specificities (Sp)  
149 of the tests used in the study were: 95.9% (Se) and 100% (Sp) for BVDV; 98.7% (Se) and 99.9% (Sp) for  
150 BHV1; 83.7% (Se) and 87.3% (Sp) for *L.hardjo*, and 64.7% (Se) and 99.2% (Sp) for MAP. These values  
151 were obtained from the manufacturers of the tests: Linnodee [*L.hardjo* ELISA (enzyme-linked  
152 immunosorbent assay)] and Pourquier (BVDV, BHV1 and MAP ELISAs). Data on the *M. bovis* status of  
153 farms was obtained from the Animal Health and Veterinary Laboratories Agency (AHVLA)<sup>1</sup>, although  
154 not all farms had whole herd tests (i.e. every animal within the farm tested) for each year of the study.  
155 Results of serological testing were reported to all farmers via their veterinarians. A farm was  
156 considered seropositive for any pathogen in any year if there was at least one test positive animal in  
157 the sample or at least one confirmed *M. bovis* reactor during an official herd *M. bovis* test. This was  
158 estimated using cattle aged between 9-21 months for BVDV, BHV1 and *L.hardjo*, to avoid interference  
159 from maternal- or vaccine-derived antibody, and cattle aged two years old and over for MAP to allow  
160 a serologic response to occur in this slowly progressing infection.

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<sup>1</sup> Now Animal & Plant Health Agency



161 *Intervention*

162 A structured approach to the provision of biosecurity advice packages was applied to each farm in the  
163 intervention group. Veterinarians developed a set of recommendations tailored to specific risk  
164 characteristics of the individual farm and farmer, informed by results of the risk assessment as well as  
165 qualitative observations including the veterinarian's perception of the farmer's behavioural  
166 characteristics and decision-making priorities. Packages could include advice that was pathogen-  
167 specific (e.g. double fencing to reduce the risk of IBR and BVD introduction) or applicable to more than  
168 one pathogen (e.g. careful sourcing of new cattle, serological monitoring and removing infected  
169 animals to reduce risk of both BVDV and MAP introduction). A strategy for the forthcoming year was  
170 discussed and agreed with the farmers, using aspirational scores to examine the potential effect of  
171 the strategy. Control farms underwent the same risk assessment. Control farmers saw the results of  
172 their risk assessment but received only general feedback and advice, within the usual scope of the  
173 veterinarian-farmer relationship, and did not examine aspirational scores or agree on a specific  
174 strategy for improvement.

175

176 *Data analysis*

177 The unit of analysis was a farm in a particular year of study, with each farm contributing four sets of  
178 data records (including the baseline data collected at the start of the project). Variations in risk scores  
179 were examined as indicators of the impact of advice packages on farmer behaviour and patterns of  
180 variation in disease measures were examined as indicators of the impact of advice on infection.  
181 Differences in risk scores and within-farm seroprevalence estimates by intervention status at the  
182 beginning (year 0) and end (year 3) of the study were examined using the Mann-Whitney U test.  
183 Differences in disease status estimates by intervention status at the beginning and end of the study  
184 were examined using the Chi-squared test. Multilevel regression analyses were used to further  
185 examine associations with intervention status and year, modification of any effect of year by  
186 intervention status (interaction), and vaccination. Potential confounding by herd size was also

187 examined. Models were built using a manual forward variable selection process with variable  
188 retention defined by  $p < 0.05$  based on the likelihood ratio statistic. Multilevel linear regression was  
189 applied to risk score data, multilevel mixed effects logistic regression for binary responses was applied  
190 to positive/negative disease status data and pathogen combinations, and multilevel logistic regression  
191 for binomial responses (number of animals testing positive as numerator and number of animals  
192 tested as denominator, with a logit link function; Stata command *melogit*) was applied to aggregated  
193 within-farm seroprevalence data (BVDV, BHV1, *L.hardjo* and MAP) to estimate the odds of  
194 seropositivity at the individual animal level. In each case, veterinary practice and farm nested within  
195 veterinary practice were examined as random effects assuming an unstructured covariance matrix. All  
196 analyses were performed using Stata/SE software version 13.1 ([www.stata.com](http://www.stata.com)). Final interpretation  
197 of results was based on a 1% significance level ( $p < 0.01$ ), to reduce the likelihood of type 1 error.

198

## 199 **Results**

### 200 *Study population*

201 During the study period, 12 (20.3%) of 59 control farms and 11 (19.3%) of 57 intervention farms were  
202 lost to follow-up, for different reasons including the discontinuation of farming. Individual veterinary  
203 practices lost between 12.5-50% of their recruited farms. By the end of the three year study period,  
204 46 intervention and 46 control farms remained in the study. Blood sampling 50 animals per farm per  
205 year regardless of farm size resulted in testing between 4% and 100% of cattle on each farm in each  
206 study year (median 32% of cattle tested per farm).

207

### 208 *Risk scores*

209 There were no significant differences in risk scores between intervention and control farms at the  
210 outset of the study, but some suggestion of a greater reduction of risk scores on intervention farms  
211 compared with control farms over the course of the study (Figure 1). Regression analyses indicated

212 significant reductions in all risk scores in years 1, 2 and 3 compared with year 0 but no significant effect  
213 of intervention status or interaction between year and intervention status (Table 1).

214

#### 215 *Farm infection status*

216 Proportions of farms identified as seropositive for each pathogen, by year and intervention status, are  
217 summarised in Table 2 and regression analyses of these relationships are summarised in Table 3. There  
218 were no significant differences in proportions of seropositive farms between intervention and control  
219 farms at the outset of the study, while at the end of the study there were significantly higher  
220 proportions of *M. bovis* positive intervention farms than control farms. During regression analyses,  
221 significantly increased odds of BVDV seropositivity were seen in association with the use of BVDV  
222 vaccination on the farm (OR 2.2;  $p=0.009$ ), but this effect was not retained in the final model because  
223 vaccination data were not available for year 0 (the reference category). There was no significant  
224 variation in odds of BVDV seropositivity by year or intervention status.

225 Proportions of farms seropositive to BHV1 varied significantly by year overall, with significantly  
226 reduced odds in year 2 compared with year 0 (OR 0.2;  $p<0.001$ ) but with no significant difference  
227 between intervention and control farms or between vaccinated and unvaccinated herds.

228 A significant interaction term in the *L.hardjo* regression model provided the most parsimonious model,  
229 but reduced odds of seropositivity on intervention farms compared with control farms at the end of  
230 the study were not significant at the 1% level. There was no significant effect of vaccination.

231 There were increased odds of MAP seropositivity in years 1 and 3 compared with year 0, with no  
232 overall effect of intervention status. No significant variation in *M. bovis* status by intervention status  
233 or by study year was identified until the addition of an interaction term, which indicated increased  
234 odds of *M. bovis* on intervention farms compared with control farms at the end of the study.

#### 235 *Apparent within-farm pathogen seroprevalence (BVDV, BHV1, L.hardjo and MAP)*

236 Within-farm seroprevalence estimates by year and intervention status are summarised in Table 4.  
237 There were no significant differences in estimates between intervention and control farms at the  
238 outset or end of the study.

239 In regression models for aggregated seroprevalence data, significant interaction terms between year  
240 and intervention status indicated significantly reduced odds of animal-level BVDV seropositivity on  
241 intervention farms compared with control farms in years 2 and 3 of the study, and of *L.hardjo*  
242 seropositivity in year 3 (Table 5). There was no significant effect of BVDV vaccination and a protective  
243 effect of *L.hardjo* vaccination did not remain significant after adjusting for either farm-level or  
244 veterinary practice-level clustering.

245 There was significant variation in within-farm BHV1 seroprevalence by year, with increased odds in  
246 year 1 and reduced odds in year 2 compared with year 0, but no effect of intervention status or  
247 vaccination and no significant intervention-year interaction. Also, increased odds of within-farm MAP  
248 seropositivity were identified in years 1 and 3 compared with year 0, with no significant effect of  
249 intervention status or significant intervention-year interaction.

250

## 251 **Discussion**

252 The impact of tailored biosecurity advice packages was assessed in these analyses by i) examining  
253 patterns of variation in risk scores, calculated through annual risk assessment interviews, as an  
254 indicator of impact on farmer biosecurity practices and ii) examining patterns of variation in farm  
255 infection status and within-farm seroprevalence, as an indicator of impact on pathogen presence.

256 This study was based on a complex data collection process, with veterinary professional opinion and  
257 trust-based relationships with farmers at its core. It is recognised that farmers are more likely to follow  
258 advice that is specifically tailored to their situation and received from someone who understands their  
259 situation (Enticott et al., 2012), and more likely to change behaviour through dialogue than instruction  
260 (Duval et al., 2016). Performing both the data collection and the intervention through the farmers'

261 regular veterinarians was therefore necessary and integral to the participatory nature of the study  
262 design, allowing assessment of what would be the gold-standard setting for such an intervention. This  
263 approach potentially introduced a source of detection bias, as veterinarians could not be blinded to  
264 the intervention status of the farm. However, as the biosecurity tool used for data collection was an  
265 objective record of farm procedures and characteristics, any such bias should be minimised. The  
266 participatory approach was also important to ensure that the annual risk assessment process resulted  
267 in a collaboratively-agreed biosecurity management strategy for the following year, thus minimising  
268 potential social desirability bias by conducting the study within the context of trust-based  
269 relationships and open discussion.

270 Regardless of intervention status, risk scores for introduction and spread of all five pathogens were  
271 significantly reduced, indicating improved biosecurity practices, in every year of the study compared  
272 with scores at the study outset. This suggests that, unless this was a consistent pattern in the wider  
273 beef industry at the time, for which we have no evidence, all farmers participating in this study, and  
274 not just those receiving specifically-tailored advice packages, were influenced by their participation.  
275 This is likely to relate to the increased level of detailed interaction between all farmers and their  
276 veterinarians during the annual risk assessment process, as well as identification of herd status in  
277 relation to endemic diseases otherwise not routinely tested for. Behaviour on control farms may have  
278 been influenced by this increased knowledge of disease status (Wolf et al., 2015), and of current level  
279 of biosecurity and the associated factors, or by provision of general biosecurity advice to control farms,  
280 possibly beyond the usual 'baseline' level, for ethical reasons, which meant that the study power to  
281 detect differences between intervention and control farms will have been lower than originally  
282 estimated using standard approaches. Furthermore, the biosecurity behaviour of control group  
283 farmers in this study is unlikely to be representative of the biosecurity behaviour of typical beef  
284 farmers in the target population, because of this increased farmer-veterinarian interaction and  
285 disease awareness, as well as participation bias relating to the type of farmer willing to enrol and  
286 remain in a longitudinal study.

287 It is encouraging, therefore, that despite these issues, there were some indications of reductions in  
288 pathogen presence on intervention farms compared with control farms in this study. Towards the  
289 end of the study period, animals on intervention farms were significantly less likely to be seropositive  
290 for BVDV or *L.hardjo* than those on control farms. It should be noted that a highly conservative  
291 Bonferroni correction for multiple tests in this study would render all relationships with pathogen  
292 presence non-significant, but given the lack of independence between tests and the number of tests  
293 conducted, this may be regarded as inappropriate (Bender and Lange, 2001). However, interpreting  
294 these results using a 1% significance level provides some, albeit not conclusive, evidence to suggest  
295 that tailored biosecurity advice packages can be effective.

296 At the lower power farm-level analysis, there was no detectable effect of the intervention on the  
297 likelihood of seropositivity to BVDV or *L.hardjo*, despite an apparent but non-significant trend for  
298 reducing BVDV seropositivity on both intervention and control farms and an interaction term in the  
299 *L.hardjo* regression model that suggested reduced seropositivity on intervention compared with  
300 control farms, but which was not significant at the 1% level. There was also no effect of the  
301 intervention on farm-level BHV1 or MAP seropositivity. The lack of any significant pattern of reducing  
302 or increasing farm-level BHV1 seropositivity through the course of the study might be explained by  
303 the nature of the immunological response to this pathogen, whereby an animal, once infected, can  
304 remain seropositive and harbour latent virus indefinitely (Jones and Chowdhury, 2007). The main  
305 focus of BHV1 infection control or reduction measures would therefore be to prevent reactivation of  
306 carriers, which would subsequently reduce virus circulation and infection of youngstock. Measures  
307 focusing on reducing pathogen introduction onto a farm through pathogen-specific biosecurity  
308 measures would be likely to have less impact. The alternative explanation for a limited impact on virus  
309 circulation is that biosecurity measures were not effective, potentially because of airborne  
310 transmission of BHV1 (Mars et al., 2000).

311 No effect of the intervention on farm-level or within-farm MAP seroprevalence was detected, with  
312 both tending to increase during the study regardless of intervention status. The most likely reason for

313 this is the complex immunology of this disease, with seropositive responses occurring after a 2-4 year  
314 incubation period (Nielsen et al., 2013).

315 The observed association of the intervention with increased likelihood of a farm being *M. bovis*  
316 positive, with intervention farms being significantly more likely than control farms to be positive at  
317 the end of the study, is difficult to explain. Confounding by geographical area should be minimal, as  
318 interventions were matched with controls within veterinary practice catchment areas. It is possible  
319 that the observation reflects changes in purchasing behaviour. For example, increased efforts to  
320 purchase animals from sources accredited as free from BVDV, BHV1, *L. hardjo* and MAP, would reduce  
321 the availability of sources that have had *M. bovis*-free status for a number of years. However, it is not  
322 possible to determine this, or explore other possibilities, using the biosecurity risk scoring tool data  
323 alone. Examination of detailed text data recorded during the annual risk assessments is required to  
324 identify strategies agreed and measures taken, which may provide some further insight into this  
325 association.

326 The biosecurity scoring tool was initially not designed to capture farm vaccination data, as vaccination  
327 does not directly prevent introduction of infection (Moennig et al., 2005; Rinehart et al., 2012;  
328 Plunkett et al., 2013; Raaperi et al., 2014). However, during discussions at the end of the first year of  
329 the study, some veterinarians commented that vaccination would be useful to record as it is used to  
330 mitigate the impact of pathogen introduction. Vaccination data were therefore recorded for the  
331 subsequent study years. The lack of complete vaccination data in year 0 precluded its useful retention  
332 in final multilevel regression models, although its effect was examined during model building, where  
333 BVD vaccination was seen to be associated with increased odds of seropositivity. As we had sampled  
334 only unvaccinated youngstock, this cannot be explained by detection of vaccine-related antibodies  
335 (Marshall et al., 1979; Savan et al., 1979; Booth et al., 2013). Instead, it is likely that farmers were  
336 responding to past BVDV incursions by adopting a vaccination strategy. The ongoing seropositive  
337 status suggests that this had not been an entirely successful measure, thus highlighting the importance

338 of a wider strategy of eliminating BVDV infection, whilst the use of vaccination is mainly to reduce the  
339 impact of BVD introduction or spread (Moennig and Becher, 2015).

340 This is the first time the biosecurity tool presented here has been used in a field study. To date, similar  
341 quantitative risk-scoring tools have been developed for poultry (Gelaude et al., 2014), in which  
342 reducing occurrence of disease outbreaks in association with increasing biosecurity is suggested  
343 (Maduka et al., 2016), and pigs, in which scores indicating better biosecurity have been associated  
344 with both improved production parameters and reduced use of antimicrobials (Laanen et al., 2013;  
345 Postma et al., 2016). In cattle, a recently published scoring system using centralised herd-level data to  
346 assess the probability of *M. bovis* infection has been proposed as means of informing risk-based  
347 trading schemes in the UK (Adkin et al., 2016).

348 Although validation of the scoring tool used in our study is ongoing, the results presented here suggest  
349 that it is a valuable, transferable framework for structuring veterinarian-farmer discussions on farm  
350 biosecurity strategies. It is plausible that the resulting increased interaction between farmers and their  
351 veterinarians, with a common focus on biosecurity, can have a beneficial impact on farmer biosecurity  
352 practices, even when not occurring to the level of the specifically-tailored biosecurity packages  
353 provided to the intervention group in this study.

354 Clearly, a key factor in the effectiveness of any advice provided by veterinarians to farmers is whether  
355 the advice is followed. The analyses presented here examine only overall patterns of farmer behaviour  
356 represented by total risk scores in each study year, and not responses to specific types of advice.  
357 Although the participatory approach used on intervention farms was designed to maximise the  
358 chances of behaviour change, it is noted that there may have been instances where strategies were  
359 agreed but not followed, which will have resulted in a further reduction of the ability of the analyses  
360 to demonstrate any impact of the intervention. Furthermore, the limited duration of the study meant  
361 that the longer-term sustainability of behaviour change could not be examined. Such sustainability is  
362 likely to be dependent on the specific biosecurity practices adopted. Further analysis of the available



363 text data would be required to inform future tailored risk scoring and strategies focusing on the types  
364 of behaviours most likely to be adopted and sustained.

### 365 **Conclusions**

366 To our knowledge, this is the first prospective intervention study to examine the effectiveness of  
367 tailored, veterinary-led biosecurity advice packages in improving farm biosecurity. For ethical reasons,  
368 the inability to completely separate intervention from control farms reduced the power of the study  
369 to detect an intervention effect and additionally, the study would have benefited from a longer  
370 duration of follow-up. Therefore, although the evidence of pathogen reduction in association with the  
371 intervention in this study is not conclusive, detection of significant reductions in *L. hardjo* and BVDV  
372 presence on intervention farms compared with control farms, suggest that such advice packages can  
373 be effective.

374 Evidence of behaviour change in all participating farmers, indicated by reducing risk scores on both  
375 intervention and control farms, are an encouraging indication that a structured annual risk assessment  
376 process, conducted as a collaboration between veterinarian and farmer, is valuable in encouraging  
377 improved biosecurity practices.

378

379

### 380 **Acknowledgements**

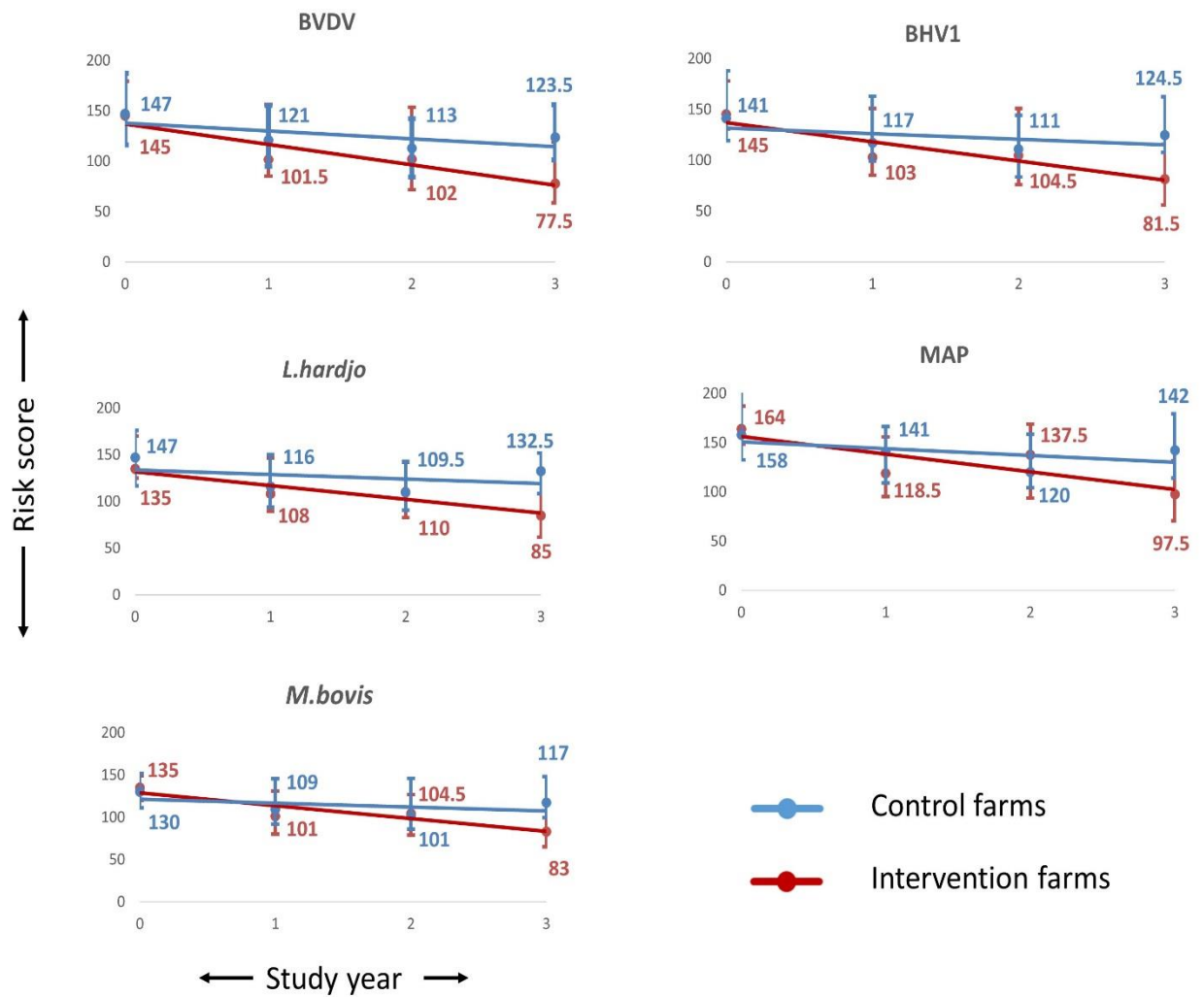
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385

386 **Conflicts of interest:** none

387

388 **Figure 1** Median risk scores for each of the five pathogens by study year and intervention status, with  
 389 fitted trendlines



390

391 Table 1: Multilevel linear regression analyses of variations in risk scores by year and intervention status, adjusted for farm-level nested within practice-level  
 392 clustering, with intervention status forced in (n=421)  
 393

		BVDV score		BHV1 score		<i>L. hardjo</i> score		MAP score		<i>M. bovis</i> score		Average score	
		$\beta$	p*	$\beta$	p*	$\beta$	p*	$\beta$	p*	$\beta$	p*	$\beta$	p*
Intervention status	Control	ref		ref		ref		ref		ref		ref	
	Intervention	-1.1	0.9	-2.76	0.9	-2.38	0.9	-2.8	0.9	-3.8	0.8	-2.56	0.9
Year of study	0	ref		ref		ref		ref		ref		ref	
	1	-25.4	<0.001	-24.3	<0.001	-20.6	<0.001	-24.0	0.001	-18.0	<0.001	-22.5	<0.001
	2	-41.0	<0.001	-39.2	<0.001	-34.2	<0.001	-38.9	<0.001	-28.0	<0.001	-36.3	<0.001
	3	-36.9	<0.001	-35.8	<0.001	-29.1	<0.001	-33.9	<0.001	-24.9	<0.001	-32.1	<0.001
Random effects variance:													
Veterinary practice		1.8e <sup>-11</sup>		1.6e <sup>-11</sup>		1.7e <sup>-9</sup>		1.7e <sup>-14</sup>		1.1e <sup>-14</sup>		9.7e <sup>-13</sup>	
Farm		7930.6		7916.6		5912.3		8339.9		4586.2		6817.7	

394 \* Wald p-value

395

396

397 Table 2: Summary of farm infection status (number and proportion of farms positive for each pathogen) by year and intervention status

		Year 0		p*	Year 1		Year 2		Year 3		p*
		n/N	(%)		n/N	(%)	n/N	(%)	n/N	(%)	
BVDV	Control	25/39	(64.1)	0.4	25/46	(54.4)	22/46	(47.8)	15/31	(48.4)	0.3
	Intervention	25/45	(55.6)		19/40	(47.5)	14/37	(37.8)	11/32	(34.5)	
BHV1	Control	12/39	(30.8)	0.1	19/46	(41.3)	9/46	(19.6)	14/31	(45.2)	0.7
	Intervention	21/45	(46.7)		12/40	(30.0)	3/37	(8.1)	13/32	(40.6)	
<i>L. hardjo</i>	Control	21/39	(53.8)	0.3	26/46	(56.5)	26/46	(56.5)	15/31	(48.4)	0.03
	Intervention	29/45	(64.4)		25/40	(62.5)	15/37	(40.5)	7/32	(21.9)	
MAP	Control	31/51	(60.8)	0.6	42/56	(75.0)	23/53	(43.4)	33/37	(89.2)	1.0
	Intervention	31/56	(55.4)		40/54	(74.1)	27/47	(57.4)	33/37	(89.2)	
<i>M. bovis</i>	Control	10/45	(22.2)	0.3	4/45	(8.9)	10/46	(21.7)	2/41	(4.9)	0.003
	Intervention	6/45	(13.3)		7/45	(15.6)	7/44	(15.9)	12/41	(29.3)	

\*Chi-2 p-value for comparison between intervention and control farms

398

399

400 Table 3: Multilevel logistic regression models for farm infection status, adjusted for farm-level nested within practice-level clustering

Risk factor	BVDV (n=317)		BHV1 (n=317)		<i>L. hardjo</i> (n=317)		MAP (n=392)		<i>M. Bovis</i> (n=352)		
	OR	p*	OR	p*	OR	p*	OR	p	OR	p*	
Intervention status	Control Intervention										
Year of study	0		ref		ref		ref				
	1		0.7		0.9		<b>2.1</b>		<b>0.01</b>		
	2		<b>0.5</b>		<b>0.2</b>		0.7		0.2		
	3		<b>0.5</b>		1.1		<b>6.1</b>		<b>&lt;0.001</b>		
Year*Intervention interaction	0 Intervention vs Control				1.6		0.3		0.5		
	1 Intervention vs Control				1.3		0.6		1.9		
	2 Intervention vs Control				0.5		0.2		0.6		
	3 Intervention vs Control				<b>0.3</b>		<b>0.04</b>		<b>8.4</b>		
Random effects variance:											
Veterinary practice		5.1e <sup>-34</sup>		7.8e <sup>-33</sup>		0.06		8.6e <sup>-37</sup>		0.7	
Farm		2.7e <sup>-34</sup>		0.2		1.33e <sup>-31</sup>		4.0e <sup>-33</sup>		1.0e <sup>-32</sup>	

401 \* Wald p-value

402

403 Table 4: Summary of within-farm seroprevalence estimates for individual pathogens by year and intervention status

		Year 0			Year 1		Year 2		Year 3		
		median	(IQR)	p*	median	(IQR)	median	(IQR)	median	(IQR)	p*
BVDV	Control	7.7	(0 – 42.1)	0.4	5.3	(0 – 50.0)	0	(0 – 52.9)	0	(0 – 38.8)	0.1
	Intervention	5.3	(0 – 28.6)		0	(0 – 55.6)	0	(0 – 9.1)	0	(0 – 6.3)	
BHV1	Control	0	(0 – 5.9)	0.2	0	(0 – 8.3)	0	(0 – 0)	0	(0 – 14.6)	0.6
	Intervention	0	(0 – 7.7)		0	(0 – 8.7)	0	(0 – 0)	0	(0 – 12.9)	
<i>L. hardjo</i>	Control	5.9	(0 – 18.8)	0.3	4.8	(0 – 11.8)	5.0	(0 – 7.1)	0	(0 – 8.4)	0.04
	Intervention	10.0	(0 – 28.6)		9.5	(0 – 16.7)	0	(0 – 5.6)	0	(0 – 0)	
MAP	Control	3.3	(0 – 4.2)	0.6	3.8	(1.1 – 11.3)	0	(0 – 3.8)	6.7	(3.3 – 16.1)	0.9
	Intervention	3.3	(0 – 7.1)		4.0	(0 – 6.9)	3.4	(0 – 6.9)	7.1	(5.7 – 13.3)	

404 IQR: bounds of interquartile range; \*Mann-Whitney p-value for comparison between intervention and control farms

405 Table 5: Multilevel logistic regression models of aggregated seroprevalence data to determine within-farm animal-level odds of seropositivity

Risk factor	BVDV (n=317)		BHV1 (n=317)		<i>L. hardjo</i> (n=317)		MAP (n=392)	
	OR	p*	OR	p*	OR	p*	OR	p*
Year of study	0		ref				ref	
	1		1.7	0.001			1.6	<0.001
	2		0.5	0.001			0.8	0.2
	3		1.2	0.2			2.6	<0.001
Year*intervention interaction	0	Intervention vs Control	0.8	0.6			1.4	0.2
	1	Intervention vs Control	0.7	0.4			1.7	0.07
	2	Intervention vs Control	<b>0.4</b>	<b>0.01</b>			0.6	0.2
	3	Intervention vs Control	<b>0.2</b>	<b>&lt;0.001</b>			<b>0.2</b>	<b>&lt;0.001</b>
Random effects variance:								
Veterinary practice			1.2 <sup>e-37</sup>		0.01		0.00002	
Farm			3.3		2.6		1.2	

406 \* Wald p-value; OR = odds of individual animal testing positive based on herd-level aggregated seroprevalence data

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