ABSTRACT

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

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

Introduction

The farmer, as a decision-maker in relation to livestock management, is a key player in the control of livestock diseases. There are many measures a farmer can take to reduce the likelihood of a pathogen being introduced and spread on the farm, which are encompassed by the broad term 'biosecurity'. Measures likely to be effective against specific cattle pathogens have been identified by risk factor studies. For example, modifiable factors such as buying in new stock and use of communal grazing are associated with increased risk of introduction of bovine viral diarrhoea virus (BVDV) to herds (Presi et al., 2011). Dias et al. (2013) found that buying in cattle, use of a bull (natural service) and renting pasture from other farmers were risk factors for bovine herpesvirus infection. Buying in cattle and the presence of other ruminants on the farm have been identified as a risk factor for *Leptospira* spp. infection (Schoonman and Swai, 2010; Williams and Winden, 2014). There is strong evidence that buying in cattle is an important risk factor for the introduction of *Mycobacterium avium subsp. paratuberculosis*, while the impact of the presence of other ruminant species is less clear (Rangel et al., 2015). Buying in cattle, long-term storage of manure and use of silage clamps have been associated with TB breakdowns (Reilly and Courtenay, 2007). Some measures, such as maintaining a closed herd or reducing contact with other animals, are therefore likely to be effective against the introduction of more than one pathogen, where transmission characteristics are shared or similar (van Schaik et al., 1999; Cowie et al., 2014; Williams and Winden, 2014).However, identification of risk factors does not equate to demonstrating effectiveness of modifying these factors in real world contexts. One challenge is that any effective biosecurity risk management strategy will usually comprise multiple components, with each component possibly contributing a relatively small effect and the relative importance of different components varying between farms. Furthermore, in order for the strategy to be implemented it must be credible to farmers and feasible in their personal context. Even then, many other factors, such as personality, experience, education (Racicot et al., 2012), perceptions, knowledge and attitudes (Toma et al., 2013; Toma et al., 2015) all play a role in determining the likely uptake of advice by farmers. Advice is more likely to be followed if it is tailored to farmers' individual contexts and characteristics rather than generic (Enticott et al., 2012; Jensen et al., 2016), and negotiated directly with them through a participatory approach (Enticott et al., 2012; Gosling et al., 2014; Duval et al., 2016) with veterinarians seen as valuable interpreters of generic advice (Garforth, 2015). Farm veterinarians, because of their knowledge of pathogens and disease as well as of the specific characteristics and circumstances of individual farms and farmers, should therefore be ideally positioned to advise effectively on individually-tailored biosecurity strategies. While it has been reported that the preferred and most influential source of advice for many farmers is their own vet (Brennan and Christley, 2013; Jones et al., 2015), it has also been acknowledged that veterinary advice is not always followed even when perceived to be useful by farmers (Brennan and Christley, 2013). Therefore, the likely effectiveness of veterinary-led individually-tailored biosecurity strategies, in terms of reduction of pathogen presence or even uptake of advice, has not been established. This intervention study was designed to assess the effectiveness of biosecurity advice packages specifically tailored to individual beef suckler farms, provided by veterinary practitioners via a participatory approach, in changing farmer behaviour and in reducing the risk of introduction or spread of infectious diseases. Objectives of the analyses presented here were (i) to identify evidence of risk behaviours changing in response to tailored veterinary biosecurity advice packages; (ii) to identify evidence of reductions in farm-level and within-farm prevalence of five important bovine pathogens: bovine viral diarrhoea virus (BVDV), bovine herpes virus-1 (BHV1, the causative agent of infectious bovine rhinotracheitis [IBR]), *Leptospira interrogans* serovar *hardjo (L.hardjo*), *Mycobacterium avium* subspecies *paratuberculosis (MAP, the causative agent of Johne's disease*), and *M. bovis* (*Mycobacterium bovis,* the causative agent of bovine tuberculosis), in association with such advice.

Materials and Methods

A randomised controlled intervention study was conducted on beef suckler farms in South West England and Wales, an area where all five infectious diseases are prevalent. Veterinarians from ten collaborating veterinary practices (one veterinarian per practice) each recruited between 8 and 18 farms, with the larger practices recruiting proportionally more farms. Inclusion criteria were that farms should have at least 30 breeding females, at least 50 overall heads of stock, a beef suckler enterprise that was more than 50% of the farm business, as well as willingness to participate. Nested within practice, farms were randomly allocated to either the intervention group, which received detailed, specifically-tailored biosecurity advice packages, or the control group, which received only generic advice provided by the veterinarian as part of the normal consultation process. Sample size estimations indicated that a total of 120 herds would be sufficient to detect a risk ratio effect of 0.32 with 80% statistical power, at a 5% significance level for a one-sided test assuming a cumulative disease incidence of 30% in the control group over the 2-year study period, and a ratio of 1:1 between controls (n=60) and intervention herds (n=60). The group size would still detect a risk ratio of 0.29 if 10% of herds within each group were lost to follow-up. In total, 57 intervention and 59 control farms were recruited. Ethical approval for the study was obtained through the Royal Veterinary College's ethical review process. Data collection began in early 2008 and finished in mid-2012.

Biosecurity scoring tool development

The ten participating veterinarians were briefed on previous work in which a farm-specific computer-based risk scoring tool for *M. bovis* had been developed (Van Winden et al., 2005; Van Winden and Aldridge, 2008). In order to create a tool capable of capturing the risks for the five specific pathogens under consideration in the current study, we adapted the evidence available at the time, which included generic risk factor categories such as cattle purchasing, direct and indirect contact with other cattle, ruminants and other animals, use of shared equipment and types of visitors to the farm, as reviewed by van Winden et al. (2005). These broad categories were divided into sub-factors to provide more detail. For example, cattle introductions were provided with details such as age, type of source (e.g. through a dealer, market, auction, etc.), number of sources and pathogen barriers provided (quarantine and testing). To elucidate risk factor weightings, the veterinarians took part in two expert opinion workshops. During the workshops, veterinarians were asked to allocate weights to reflect the relative importance of specific sub-factors, such that the total weight of all sub-factors with each broad risk factor category would be 100%, in an approach similar to that used in the Competing Values Framework (Cameron and Quinn, 2006). In addition, veterinarians evaluated the importance of herd size for risk of pathogen introduction and spread. A semi-Delphi approach was used in order to achieve near-consensus (Gallagher, 2004; Banwell et al., 2005). After the first workshop the median of the proposed weights for each risk factor was presented and discussed in the second workshop. After the second workshop the veterinarians completed the process once again and the medians of these weights were subsequently used to construct the scoring tool in Microsoft Excel™, with creation of an algorithm to generate higher scores for farms with higher risk for disease introductions or spread and a lower scores for more biosecure units. Herd size weightings were used to create a curvilinear multiplier which generated increasing scores with increasing animal numbers. The final version of the scoring tool, including the median weightings for each risk factor, is presented in the supplemental material. The overall biosecurity score is the sum of factors contributing to the overall risk and the spreadsheet identifies the main risk contributor. This allowed farmers and veterinarians to identify specific factors that could be targeted for change during the following year, and by altering these factors an aspirational score could be generated. Before the scoring tool was used on the farms, training was provided for participating veterinarians, to familiarise them with the spreadsheet and to address any concerns.

Data collection

Risk measures:

Veterinarians visited all farms annually to complete the risk assessment questionnaire, recording existing biosecurity practices, resulting in 4 risk assessments per farm. In the analyses presented here, year 0 therefore represents the data collected at the start of the study and years 1-3 represent data collected at the end of year 1, 2 and 3 of the study. Initially, farm vaccination history was not recorded, but following discussions with participating veterinarians at the end of year 1, farm-level vaccination status for BVDV, BHV1 and *L.hardjo*, reflecting relevant vaccinations in the previous year, were recorded thereafter.

Indicators of pathogen presence:

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

[1] Now Animal & Plant Health Agency

Intervention

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

Data analysis

The unit of analysis was a farm in a particular year of study, with each farm contributing four sets of data records (including the baseline data collected at the start of the project). Variations in risk scores were examined as indicators of the impact of advice packages on farmer behaviour and patterns of variation in disease measures were examined as indicators of the impact of advice on infection. Differences in risk scores and within-farm seroprevalence estimates by intervention status at the beginning (year 0) and end (year 3) of the study were examined using the Mann-Whitney U test. Differences in disease status estimates by intervention status at the beginning and end of the study were examined using the Chi-squared test. Multilevel regression analyses were used to further examine associations with intervention status and year, modification of any effect of year by intervention status (interaction), and vaccination. Potential confounding by herd size was also examined. Models were built using a manual forward variable selection process with variable retention defined by $p<0.05$ based on the likelihood ratio statistic. Multilevel linear regression was applied to risk score data, multilevel mixed effects logistic regression for binary responses was applied to positive/negative disease status data and pathogen combinations, and multilevel logistic regression for binomial responses (number of animals testing positive as numerator and number of animals tested as denominator, with a logit link function; Stata command *melogit*) was applied to aggregated within-farm seroprevalence data (BVDV, BHV1, *L.hardjo* and MAP) to estimate the odds of seropositivity at the individual animal level. In each case, veterinary practice and farm nested within veterinary practice were examined as random effects assuming an unstructured covariance matrix. All analyses were performed using Stata/SE software version 13.1 (www.stata.com). Final interpretation of results was based on a 1% significance level ($p<0.01$), to reduce the likelihood of type 1 error.

Results

Study population

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

Risk scores

There were no significant differences in risk scores between intervention and control farms at the outset of the study, but some suggestion of a greater reduction of risk scores on intervention farms compared with control farms over the course of the study (Figure 1). Regression analyses indicated significant reductions in all risk scores in years 1, 2 and 3 compared with year 0 but no significant effect of intervention status or interaction between year and intervention status (Table 1).

Farm infection status

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

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

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

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

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

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

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

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

Discussion

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

This study was based on a complex data collection process, with veterinary professional opinion and trust-based relationships with farmers at its core. It is recognised that farmers are more likely to follow advice that is specifically tailored to their situation and received from someone who understands their situation (Enticott et al., 2012), and more likely to change behaviour through dialogue than instruction (Duval et al., 2016). Performing both the data collection and the intervention through the farmers' regular veterinarians was therefore necessary and integral to the participatory nature of the study design, allowing assessment of what would be the gold-standard setting for such an intervention. This approach potentially introduced a source of detection bias, as veterinarians could not be blinded to the intervention status of the farm. However, as the biosecurity tool used for data collection was an objective record of farm procedures and characteristics, any such bias should be minimised. The participatory approach was also important to ensure that the annual risk assessment process resulted in a collaboratively-agreed biosecurity management strategy for the following year, thus minimising potential social desirability bias by conducting the study within the context of trust-based relationships and open discussion.

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

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

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

No effect of the intervention on farm-level or within-farm MAP seroprevalence was detected, with both tending to increase during the study regardless of intervention status. The most likely reason for this is the complex immunology of this disease, with seropositive responses occurring after a 2-4 year incubation period (Nielsen et al., 2013).

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

The biosecurity scoring tool was initially not designed to capture farm vaccination data, as vaccination does not directly prevent introduction of infection (Moennig et al., 2005; Rinehart et al., 2012; Plunkett et al., 2013; Raaperi et al., 2014). However, during discussions at the end of the first year of the study, some veterinarians commented that vaccination would be useful to record as it is used to mitigate the impact of pathogen introduction. Vaccination data were therefore recorded for the subsequent study years. The lack of complete vaccination data in year 0 precluded its useful retention in final multilevel regression models, although its effect was examined during model building, where BVD vaccination was seen to be associated with increased odds of seropositivity. As we had sampled only unvaccinated youngstock, this cannot be explained by detection of vaccine-related antibodies (Marshall et al., 1979; Savan et al., 1979; Booth et al., 2013). Instead, it is likely that farmers were responding to past BVDV incursions by adopting a vaccination strategy. The ongoing seropositive status suggests that this had not been an entirely successful measure, thus highlighting the importance of a wider strategy of eliminating BVDV infection, whilst the use of vaccination is mainly to reduce the impact of BVD introduction or spread (Moennig and Becher, 2015).

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

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

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

Conclusions

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

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

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Conflicts of interest: none

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

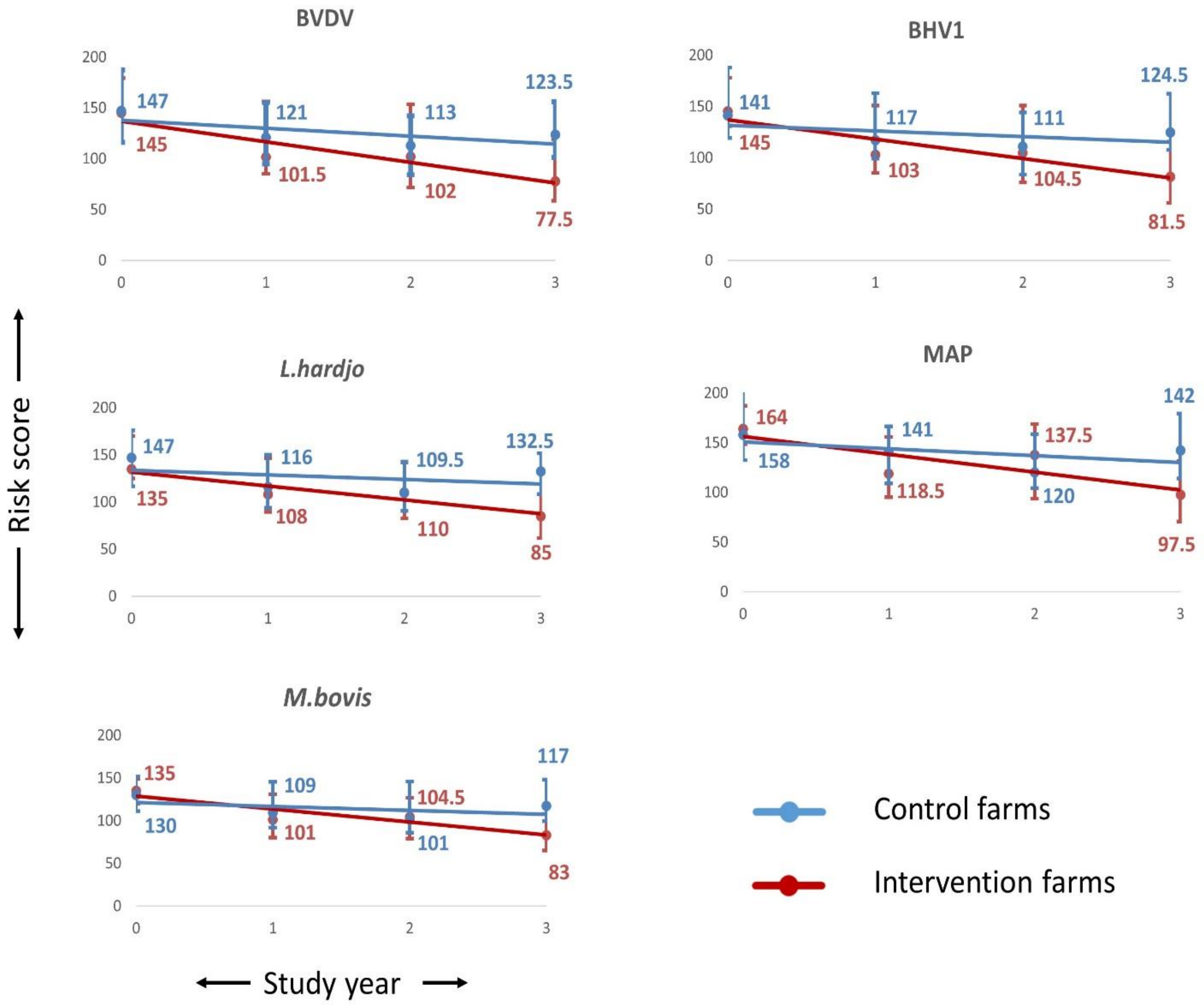

Table 1: Multilevel linear regression analyses of variations in risk scores by year and intervention status, adjusted for farm-level nested within practice-level clustering, with intervention status forced in (n=421)

		BVDV score		BHV1 score		*L. hardjo* score		MAP score		*M. bovis* score		Average score	
		ß	p*	β	p*	β	p*	β	p*	β	p*	β	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		$1.8e^{-11}$		$1.6e^{-11}$		$1.7e^{-9}$		$1.7e^{-14}$		$1.1e^{-14}$		$9.7e^{-13}$	
Farm		7930.6		7916.6		5912.3		8339.9		4586.2		6817.7	

* Wald p-value

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

		Year 0			Year 1		Year 2		Year 3		
		n/N	(%)	p*	n/N	(%)	n/N	(%)	n/N	(%)	p*
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)	
L. hardjo	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)	
M. bovis	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

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

Risk factor			BVDV (n=317) OR	BVDV p*	BHV1 (n=317) OR	BHV1 p*	*L. hardjo* (n=317) OR	*L. hardjo* p*	MAP (n=392) OR	MAP p	*M. Bovis* (n=352) OR	*M. Bovis* p*
Intervention status	Control											
	Intervention											
Year of study	0		ref		ref				ref			
	1		0.7	0.3	0.9	0.7			**2.1**	**0.01**		
	2		**0.5**	**0.04**	**0.2**	**0.001**			0.7	0.2		
	3		**0.5**	**0.02**	1.1	0.7			**6.1**	**<0.001**		
Year*Intervention interaction	0	Intervention vs Control					1.6	0.3			0.5	0.2
	1	Intervention vs Control					1.3	0.6			1.9	0.3
	2	Intervention vs Control					0.5	0.2			0.6	0.4
	3	Intervention vs Control					**0.3**	**0.04**			**8.4**	**0.009**
Random effects variance:												
Veterinary practice			$5.1e^{-34}$		$7.8e^{-33}$		0.06		8.6^{e-37}		0.7	
Farm			$2.7e^{-34}$		0.2		1.33^{e-31}		4.0^{e-33}		1.0^{e-32}	

* Wald p-value

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)	
L. hardjo	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)	

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

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)		*L. hardjo* (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	**0.4**	**0.01**			0.6	0.2		
	3	Intervention vs Control	**0.2**	**<0.001**			**0.2**	**<0.001**		
Random effects variance:										
Veterinary practice			1.2^{e-37}		0.01		0.00002		8.9^{e-33}	
Farm			3.3		2.6		1.2		0.2	

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

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