Field trials and tribulations: mortality, morbidity and liveweight following multivalent clostridial and Pasteurella vaccination of lambs on six English commercial sheep flocks

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ABSTRACT

Objective: This field trial aimed to assess the effect of a multivalent clostridial and *Pasteurella* vaccine (Heptavac P Plus, MSD Animal Health, 2015), administered to lambs at two different time points, on lamb mortality (primary outcome), morbidity and growth rates (secondary outcomes) as compared to unvaccinated lambs.

Background: This veterinary practice-based study was motivated by a knowledge gap identified during flock health planning activities and engagement between veterinarians and commercial sheep producers in a regional knowledge exchange programme in South West England. Common queries to the veterinary practice from sheep producers during summer to autumn 2012 included the value and timing of vaccinations to prevent disease associated with pasteurellosis. Discussions between veterinarians and consultants working in preventive sheep flock health planning stimulated a scientific literature search, which highlighted the lack of published data on field vaccine testing of *Pasteurella* components under British sheep commercial farming systems, and a lack of strong evidence in order to inform practical questions regarding the optimal timing of vaccinations aimed at preventing lamb mortalities in the pre-, peri- and post-weaning periods.

Evidentiary value: A field vaccine trial was conducted on six commercial sheep flocks in South West England. From April 2013, across the six farms a total of 900 twin-born lambs (*Ovis aries*) were systematically randomly allocated into 1. unvaccinated, 2. early- (6 to 8-week-old) or 3. late- (18 to 20-week-old) vaccination groups. The study provides evidence to support recommendations for sheep producers regarding risks for clostridial disease in fully unvaccinated sheep flocks, supports the benefits of preventive ewe vaccination, and indicates that pre-weaning vaccination of lambs may be beneficial for reducing peri- and post-weaning losses on some flocks. The study highlights the challenges of identifying the reasons for mortality in grazing lambs, and provides new evidence to support the need for early intervention and treatment of ocular lesions in young lambs, in order to reduce their negative impact on lamb performance.

Methods. During the 6-month trial, lambs were assessed for ocular lesions, orf lesions, clinical respiratory disease, lameness, diarrhoea, and ear tag losses. Monthly liveweights (kg) and mortalities were recorded. Lambs were removed from the study when they reached producer-defined finishing objectives, ≥46 kg liveweight or when they died.

Results: Overall, low levels of mortality and disease outcomes were observed in both control and vaccinated lambs. A mortality rate of 33 deaths per 1000 lambs at-risk, 0.8% lameness (95% CI 0.6–1.1), 1.7% diarrhoea (95% CI 1.4–2.2), 3% ocular disease (including ocular discharge and/or entropion) (95% CI 2.2–3.2), and 2.6% orf lesions (95% CI 2.2–3.1) were recorded over the trial period. A higher proportion of mortalities occurred in unvaccinated or partially vaccinated lambs (n=23; 76.7%), as compared to trial lambs that had received a primary vaccine course (n=7; 13.3%). A small proportion of mortalities (n=3; 10.0%) occurred in lambs whose vaccination status could not be confirmed No clinical signs of respiratory disease were recorded during veterinary or producer assessments of control or vaccinated trial lambs. Mixed-effects models found significant between-farm and time differences in liveweights (p<0.001) but no significant effect of vaccination status on lamb growth rates. A dramatic decrease in lamb growth rates across all farms coincided with the weaning period – this was also the period in the study were the majority of grazing lamb mortalities were identified in flocks that routinely vaccinated their ewes against clostridial disease and pastuerellosis.

Conclusion: A clear trend was found with fewer mortalities occurring in lambs that received a multivalent clostridial and *Pastuerella spp*. vaccination course compared to unvaccinated lambs. The reasons behind mortalities in grazing lambs were not diagnosed given the low submission of carcases for veterinary necropsy examination that was attributed to carcase scavenging and decomposition. No significant effects of vaccination were found on lamb growth rates or clinical outcomes. However, a secondary finding was the significant negative effect of ocular conditions (including discharge and entropion) on growth rates of lambs

aged 6 weeks and over – supporting the need for early recognition and intervention in order to reduce animal welfare and production impacts.

Application: We highlight study findings, field experiences and discuss some of the practical challenges and considerations in design and conduct of field vaccine trials used to inform evidence-based veterinary practice. The study will be of interest to veterinary surgeons, sheep producers, flock health and agricultural consultants, pharmaceutical agencies and advisors, researchers and those engaged in applied studies in animal health and welfare, veterinary epidemiology, preventive health management researchers, and participants and advisors in field vaccine trial design and development.

INTRODUCTION

Clostridial diseases and pasteurellosis are endemic diseases of sheep and reported to be among the most common causes of mortality in growing lambs (Lewis, 2007). *Clostridia* are commensals of the gastrointestinal tract (Uzal, 2004) and are found widely in both soil and water sources (Al Saif and Brazier, 1996), whilst *Mannheimia haemolytica* and *Bibersteinia trehalosi* are commensals of the ovine upper respiratory tract of healthy sheep (Donachie, 2007). Clostridial disease and pasteurellosis are both believed to be caused by 'stressor' or 'trigger' factors, such as sudden weather changes, abrupt dietary change or movement, all of which can facilitate rapid bacterial multiplication, toxin production and commensal tissue invasion. Disease outbreaks are frequently unpredictable, and progression can be so rapid that they present as 'sudden deaths' (Donachie, 2007)*.

Both experimental and field research studies have identified that multivalent vaccines provide sheep with effective *Clostridia spp*. antibody responses (Brown et al., 1976; Wells et al., 1984; and Green et al., 1987). Consequently, prophylactic flock vaccination is considered to be key to clostridial disease control and prevention. Mortalities associated with clostridial diseases are most frequently reported in unvaccinated sheep flocks or in those not fully adhering to recommended vaccination regimes (Lewis, 2007). In comparison to the evidence supporting clostridial *spp*. vaccination, there is much weaker evidence regarding the *Pasteurella* component efficacy. The efficacy of *Pasteurella spp*. vaccination was originally tested in laboratory challenge studies on experimentally-infected, specific-pathogen-free lambs (Gilmour et al., 1979; Wells et al., 1979: Jones et al., 1989). No effect of a combined *Pasteurella spp.* vaccine was found on the level of pneumonic lesions found in lambs when compared to unvaccinated control lambs (Chandrasekaran et al., 1991). Similarly, field vaccination of Bighorn ewes following a pneumonia outbreak with either an experimental *Birbersteinia trehalosi* and *M. haemolytica* vaccine or a commercial *Pasteurella multocida* and *M. haemolytica* bovine vaccine provided no beneficial effect on neonatal lamb survival (Cassirer et al., 2001). Furthermore, field research into an ovine *Pasteurella* vaccine on commercial New Zealand flocks identified no significant differences in lung lesion scores between vaccinated and unvaccinated lambs (Goodwin-Ray et al., 2008). The lack of proven field efficacy of commercially available *Pasteurella spp*. ovine vaccines in New Zealand has stimulated experimental research into the cross-protective efficacy of a subcutaneously administered *M. haemolytica* S1 strain (Zheng et al., 2015). Development of effective vaccines to prevent pasteurellosis appears to be challenging given the interplay between bacteria, including *Pasteurella spp*. with Mycoplasma *spp*. and viruses that commonly occur (Malone et al., 1988).

Currently, strong evidence regarding field efficacy and immunity derived from *M. haemolytica* and *B. trehalosi* components of multivalent *Clostridia* and *Pasteurella* vaccines as tested in British commercial sheep production systems is lacking. This study, therefore, aimed to capture novel field data to inform veterinary flock health planning, specifically regarding best practice administration and timing of vaccinations aimed at reducing pasteurellosis on six commercial sheep farms.

Inclusion criteria

A convenience sample of six sheep flocks in North Devon, England (clients located within a 15 mile radius of Torch Farm and Equine Ltd., South Molton, a veterinary practice) were recruited. Farms eligible for this study were commercial spring-lambing flocks with a minimum of 250 breeding ewes producing either slaughter lambs or breeding replacements (Table 1); that were not currently vaccinating lambs against *Clostridia spp.* or *Pasteurella*; and had a history of sudden death in lambs with confirmed or suspected pasteurellosis (based on prior veterinary diagnostic investigations including post-mortem examination). Prior to enrolment, the veterinarian (MJG) and researcher (CJP) visited farms to gather background data on flock management, disease control practices, and to assess eligibility criteria. Informed and written producer consent was obtained. Farms were provided with a study protocol, and a mortality and treatment recording book.

Table 1 Study farm details

Farm ID	Production objectives	Farm type	Ewe breeds	Ram breeds	n breeding ewes at 2012 mating	Peak 2013 lambing date	Approximate lamb age (weeks) at study entry	n lambs recruited
1	Lamb finishing	Lowland	North Country Mule, Texel-cross	Texel	1269	28 February	8	135
2	Lamb finishing	Hill (SDA)	Highlander	Primera	1176	25 March	6	135
3	Breeding and lamb finishing	Lowland	North Country Mule, Suffolk-cross Mule	Charollais, Abermax, Suffolk	740	3 April	8	135
4	Breeding and lamb finishing	Hill (SDA)	North Country Mule	Cheviot, Berrichon du cher, Texel, Charollais	790	2 April	8	135
5	Breeding and lamb finishing	Hill (SDA)	Lleyn, North Country Mule	Lleyn and Charollais	1046	18 April	6	135
6	Breeding and lamb finishing	Upland & Hill (SDA)	Exlana, Lleyn, Lleyn-cross	Exlana	1420	28 April	8	225

SDA: Severely disadvantaged area

Sample size

In the absence of any known prevalence data, sample size estimates were based on prior veterinary knowledge of diseases in the recruited flocks and ability of the study to detect an assumed 3% difference in mortality (primary outcome) and morbidity (secondary outcome) between early and late vaccinated lambs. Sample size calculations assumed that the minimum level of mortality was 3%, the assumed relative risk of disease in the unvaccinated group was 3: the Type 1 error probability was set at α = 5% and the power of the study was (1-β) = 80%. This required a sample size of 243 individuals in each group, giving a total of 486 lambs. To maintain disease challenge and reduce the possible effect of confounding from protection afforded by early vaccinated lambs, a further group of lambs was included in the trial and remained unvaccinated as per standard farm policy. One farm that had been recruited unexpectedly dropped out shortly before study commencement, and, as a consequence, a later lambing flock that met the eligibility criteria was recruited (farm 6) as a replacement.

To limit the impact of further farm drop-out or unexpected disease outbreaks, a slightly larger than estimated sample size was included. Accordingly, a total of 900 twin lambs of various breeds (Table 1) aged between 6 and 8 weeks (based on peak lambing dates) were enrolled into the trial.

Study design

Heptavac P Plus (MSD Animal Health, 2015) is marketed as a preventive vaccine for reducing flock mortalities and morbidity associated with seven specific clostridial diseases and for the control of pneumonic pasteurellosis (*M. haemolytica)* in lambs from a minimum age of 3 weeks, and in the control of systemic pasteurellosis *(Bibersteinia trehalosi)* in weaned fattening and breeding sheep (MSD Animal Health, 2015). The objective of the field trial was to assess the effect of this multivalent *Clostridia spp*. and *Pasteurella* vaccine administered to lambs from six commercial sheep flocks at early (6 to 8 weeks old) or late post-weaning (18-20 weeks old) vaccination time points on lamb mortality (primary outcome), morbidity and growth rate (secondary outcomes).

To reduce within-farm genetic, management and nutritional effects, only twin lambs were included. The sample comprised 112 entire rams (12.4%), 322 castrated ram (35.7%) and 466 ewe (51.8%) lambs of a variety of breeds presented by the producer (Table 1). Systematic random allocation of trial lambs into either unvaccinated, early or late (post-weaning) vaccination groups was applied by the researcher (CJP) according to the order that the lambs walked into the handling race. Lambs were not matched on sex or breed.

At visit 1, lambs were individually ear tagged by the producer. Due to limited study resources and aims to keep lambs managed as per the usual routines of each farm, producers inserted their regular ear tag of choice. Whilst the selection of tag brand was not part of the study design, with the exception of farm 3, single tagging and the same tag brand was used on all farms. At each visit, lambs were firstly individually examined for six standardised clinical descriptors (Table 2). The same experienced veterinarian (MJG) performed examinations on all farms, and remained blinded to data captured from other study visits. Lambs were assessed whilst moving and standing in the handling race, prior to entering the weighing crate and before vaccination. At each visit, farm weighing scales were calibrated before individual liveweight data (kg) capture. The researcher maintained data entry.

On each farm, all trial lambs (including early and late vaccinated lambs and unvaccinated lambs) were managed as a single management group until weaning, when they were separated into two same-sex management groups. Trial lambs were managed as a separate group and did not graze with other (non-trial) lambs on the farm from the period of study, enrolment to end. Lambs were selected for slaughter according to producer preference for weight, carcase conformation and fat class; if selected prior to the study visit, lambs were weighed the day before slaughter by the producer.

All vaccinations were performed by the same veterinary surgeon following manufacturer (MSD, 2015 and best practice guidelines on vaccine storage and administration industry (RUMA, 2006). As illustrated in Fig. 1, lambs were vaccinated with a primary course of Heptavac P Plus at two time points: 'early' (first vaccine administered when lambs were approximately 6 to 8 weeks-old) and 'late' (first vaccine administered when lambs were approximately 18 to 20-weeks-old). Primary vaccination consisted of two doses (2 ml) of Heptavac P Plus injected subcutaneously 5–10 cm below the ear using a sterile vaccination system (Sterimatic Worldwide Ltd, UK). Dosing interval was 4 to 5 weeks. Vaccine was stored at 4°C and transported to farms in insulated cool boxes. Bottles were unopened until just before administration. A new vaccination bottle was used for each farm, and once opened, vaccine was used within 2 hours. The veterinarian who administered all vaccinations was not blinded to vaccine type or brand. No placebo was used. The researcher sprayed the fleece of all trial lambs with a colour mark based on allocation group; colours differed between study farms. The veterinarian and producer were not informed of fleece codes. Due to the on-farm nature of the trial and their necessary involvement with handling and vaccinating lambs and need for informed consent and awareness of the vaccine treatment groups, they were not considered to be fully blinded. At each visit, farmer-

maintained mortality and treatment records were collected. Throughout the trial, farms were asked to present trial and non-trial lamb mortalities for necropsy examination.

Table 2 Standardised clinical descriptors for assessment of lamb health and morbidity (secondary outcome) scored on binary scale (absent/present)

Clinical outcome	Assessment criteria
Clinical respiratory signs	Observed signs of mucopurulent nasal discharge, and/or persistent severe coughing, and/or dyspnoea and/or, tachypnoea
Facial and oro-nasal orf lesions[a]	Observed active and healing facial and oro-nasal orf lesions: external lesions, with or without discharging pustules and/or associated signs of inflammation; significant swelling and hyperaemia, and/or without signs of keratinization[a]
Ocular discharge/lesion/entropion[b]	Observed ocular discharge (purulent, or haemorrhagic, or tear staining) and/or keratitis, conjunctivitis, and/or corneal ulcer and/or entropion
Ear tag lesion	Observed external lesions (abscesses and/or purulent discharge and/or tears and/or rips) around site of ear tag
Lameness[b]	Observed three-legged gait and/or holding a foot off the ground and/or stiff or stilted gait, and/or head-nod associated with gait
Diarrhoea[b]	Observed diffuse faecal perineal soiling

[a] Scored as per descriptors of Lovatt et al. (2012)
[b] Scored as per descriptors of Phythian et al. (2012)

Trial time schedule is illustrated in Fig. 1. Early vaccinated lambs received their first primary dose (aged approximately 6 to 8 weeks-old) at visit 1 and the second 1 month later (visit 2). On farms 1 to 5, lambs were weaned prior to visit 4; late vaccinated lambs received their first primary dose at visit 4 (approximately 18 to 20 weeks old) and second dose at visit 5. Due to management constraints and later recruitment, farm 6 lambs were weaned just prior to visit 3. Late vaccination was conducted at visits 3 (when lambs were approximately 16 weeks old) and 4. Due to the early slaughtering of lambs on farm 1, only two late vaccine group lambs received the full vaccine course. The study ended on 7 November 2013 before trial lambs reached finishing objectives and exclusion weight criteria on farm 6.

Farm ID	Month							
	April	May	June	July	August	September	October	November
Farm 1	1st	2nd		W / 1st	2nd [a]	End		
Farm 2		1st	2nd	W	1st	2nd	End	
Farm 3		1st	2nd	W	1st	2nd	End	
Farm 4		1st	2nd	W	1st	2nd	End	
Farm 5		1st	2nd	W	1st	2nd		End
Farm 6			1st	2nd	W	1st	2nd	End

☐ Early vaccine   W Weaning   ▨ Late vaccine   ■ End Trial   [a] n=two lambs

Figure 1 Schedule of timings for the first (1st) and second (2nd) vaccinations of the primary vaccine course for early and late vaccination groups, weaning and the end of trial on each of the six study flocks

Ethical approval

The study was part of recognised veterinary practice under the Veterinary Surgeons Act of 1966. Ethical approval was obtained from the University of Bristol ethics committee (ethical review reference VIN/13/021).

Statistical analysis

Data was entered into Excel (Microsoft Office) and analysed using Stata version 13 (Statacorp, Texas). Based on the study start date, study age (days) was assigned for each weight-recording session. Since these commercial farms had slightly different finishing goals, if lambs had not already left the trial, the exclusion criteria and final visit liveweight was set as ≥46 kg. Descriptive statistics explored the number of lambs, and proportion of mortality, morbidity, tag losses, and liveweights on each farm at each study period. One-way analysis of variance (ANOVA) examined for significant differences in mean liveweight of each group at study entry, in order to assess for any systematic bias in allocation. Differences in overall mortality rates between vaccinated and unvaccinated trial lambs were examined for significance using Wilcoxon signed-rank (paired) test. Growth rates (daily liveweight gain – DLWG, g/day) were calculated between successive paired visits as: difference in weight/number of days between successive weighings. Overall, mean trial lamb DLWG (W0) was calculated as: (last recorded weight minus weight on visit 1 / number of days in trial)*1000. Mean difference in liveweight gain (kg) between unvaccinated and early vaccinated lambs was examined from visit 1 up to visit 4 (weaning) using paired t-tests. Statistical significance testing was set at $p \leq 0.05$.

Data modelling was supported by a statistician (PJC) blinded to vaccine status. Firstly, using mixed effects multilevel linear regression models including farm identity and visit number (1 to 6) as fixed effects to assess the effect of vaccination status (unvaccinated, early or post-weaning vaccination) on mortality, liveweight and DLWG. The continuous predictor 'study age' was also forced into all models to assess the effect of vaccination status on DLWG. No significant interaction terms were identified. Later, farm identity was included as a random effect, as individual lamb identity was nested within farm. Visit period (1 to 6) was also examined as a random effect. Residuals were modelled in an autoregressive structure with an order of one. Effect of lameness, ocular condition, orf lesion, diarrhoea or ear tag lesion on liveweight were analysed in separate mixed-effects regression models.

During analysis, farm 1 lambs originally allocated into the late group but that were not vaccinated were recoded as unvaccinated lambs. Similarly, farm 6 entered the trial later, and the trial ended before lambs

reached finishing objectives/exclusion criteria. Therefore, two datasets – 1. entire dataset of all six farms and 2. dataset with removal of farms 1 and 6 – were compared to assess for significant differences in study findings.

To assess the effect of vaccination up to the point of weaning (i.e. early vaccination versus no vaccination), lambs originally allocated to the late vaccination group were recoded as unvaccinated. Fixed and mixed-effects multilevel linear regression models, as described above, were used to identify any differences between liveweight and DLWG of unvaccinated and early vaccinated lambs up to visit 4 on farms 1 to 6.

RESULTS

Farm management

With the exception of farm 1, all other study flocks had at least a 5 year history of prophylactic administration of multivalent combined clostridial and *Pasteurella* vaccine (Heptavac P Plus, MSD Animal Health, 2015) to breeding animals (primary course and a booster 4 to 6 weeks prior to expected peak lambing date). No flocks had administered multivalent clostridial or *Pasteurella* vaccines to pre-weaned lambs in the previous 5 years. All lambs were routinely tail-docked using rubber rings within the first week of life and, except for farm 6, routinely castrated with rubber rings. On farm 5, all lambs routinely received a live orf vaccine (Scabivax Forte, MSD Animal Health, 2015) prior to turnout onto pasture. Trial lambs received oral anthelmintics, on average five times (range 4–6) during the trial period. Farm 1 employed a rotational grazing system; farm 2 moved stock every 3 days, whilst farms 3 to 6 used set-stocking. On all farms, trial lambs were maintained as a single management group. On each farm, all trial lambs were grazed together as a single management group until weaning, when male and female trial lambs were separated and managed as two same-sex trial groups. Farm 1 provided creep feeding for lambs from visit 1; farms 2 to 5 provided supplementary creep feed from weaning onwards; and farm 6 provided no concentrates during the study.

Animals

Between 23 April and 7 November 2013, the number of lambs assessed per visit decreased over time, reflecting cumulative effects of mortalities (Table 3 and Table 4), exclusion of three sick lambs between visits 4 and 5 (fractured limb, blind, and recumbent) and lambs reaching finishing or exclusion criteria from visit 3 onwards. Overall, 72 tag losses (8.0%) were recorded (Table 3). Due to double ear-tagging on farm 4, only 52 replacement tags were inserted.

Table 3 Number of lambs recruited, recorded ear tag losses, lamb mortalities, earliest and latest finishing times and minimum weight of finished trial lambs on six sheep flocks

Farm ID	n lambs recruited to study	n (%) ear tag losses in live lambs	n (%) trial mortalities recorded	n (%) mortalities confirmed in unvaccinated trial lambs[a,b]	n (%) mortalities confirmed in vaccinated lambs[b]	n (%) unconfirmed vaccination status [c]	Earliest finishing time from study entry (study age)	Minimum weight of finished trial lambs (kg)	Latest finishing time from trial entry (study age)
Farm 1	135	21 (15)	7 (5.2)	7 (100)	0 (0)	0 (0)	56 days	40	148 days
Farm 2	135	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	90 days	41.5	154 days
Farm 3	135	36 (26.7)	4 (3.0)	2 (50.0)	2 (50.0)	0 (0)	122 days	43	155 days
Farm 4	135	5 (3.7)	7 (5.2)	6 (85.7)	0 (0)	1 (14.3)	125 days	42	154 days
Farm 5	135	4 (3.0)	3 (2.2)	2 (66.7)	0 (0)	1 (33.3)	99 days	42.4	162 days
Farm 6	225	6 (2.7)	9 (4.0)	6 (66.7)	2 (33.3)	1 (11.1)	[d]	[d]	[d]
Total	900	72 (8.0)	30 (3.3)	23 (76.7)	4 (13.3)	3 (10.0)			

[a] Unvaccinated lambs include those in the unvaccinated trial group and trial animals in early or late vaccination groups prior to completion of the primary vaccination course following vaccine datasheet guidance.
[b] Trial lambs were considered to be fully vaccinated two weeks following the correct administration of the primary vaccine course) in adherence with vaccine datasheet guidance.
[c] Due to timing of death occurring between visit dates and lack of an exact dead date it is unclear whether early vaccine group lambs were fully vaccinated according to datasheet guidance at the time of death.
[d] Lambs did not reach producer finishing target or reach study exclusion criteria before the end of the study period.

Mortality

A total of 30 mortalities (3.3%) were recorded in trial lambs, which was equivalent to a study mortality rate of 33 deaths per 1000 lambs at-risk. Ear tags were retrieved by producers from 14 trial lamb carcases and presented to the researcher, allowing full confirmation of the individual lamb identity in these cases. For the remaining mortalities, trial group status was identified by examining database records and cross-checking the number of lambs at each farm visit. Lambs were presumed dead if they were missing on at least two consecutive visits, and had not been slaughtered. This approach permitted confirmation of the group and vaccination status of all but three mortalities (Table 3).

Mortalities arose in both vaccinated and unvaccinated trial lamb groups (Table 3). A clear trend was found with a higher level of lamb losses recorded in unvaccinated lambs, compared to lambs that were considered to be fully vaccinated (p=0.052). Overall, farms 1 and 4 experienced the highest levels of trial lamb mortality (5.2%). On farm 1, all mortality cases occurred in unvaccinated lambs or before primary vaccine course completion. Due to the lack of an exact death date for some lambs, it was not possible to confirm whether three trial lambs in the early vaccinated group were considered to be fully vaccinated (Table 3). Overall, 14 trial lambs died pre-weaning and 16 trial lambs died in the period post-weaning. There was some between-farm variation in the timing of lamb deaths. All trial lamb losses (n=7) on farm 1 occurred from early June to early July, i.e. before weaning (Fig. 1), although lambs on this farm were receiving concentrate feed at their time of death. By contrast, there were two main periods for the timing of lamb losses (n=23) on farms 3–6. Deaths of grazing lambs on farms 3–6 mainly occurred in the week of and up to 1 month post-weaning (16/23, 69.6%), i.e. late July to late August. The remainder of trial deaths on farms 3–6 occurred pre-weaning and were concentrated in the late June to late July period (7/23, 30.4%).

The reasons for mortalities in trial lambs was not fully elucidated due to the very low number (n=2) of animals presented for veterinary post-mortem examination; both lamb carcases examined came from a single study flock. On farm 1, one trial lamb from the unvaccinated group (approximately 4 months old) was presented for necropsy on 13 June 2013 at the veterinary practice, and an unvaccinated non-trial lamb (coinciding with reported 'sudden deaths' in eight non-trial lambs), was necropsied on 28 June 2013 (MJG) during a trial farm visit. Both lambs were in good body condition. Gross post-mortem findings in the trial lamb included the presence of blood-tinged frothy fluid present in the trachea and congestion of both lung lobes with frothy fluid. The thymus surface and thoracic fat surface were petechiated, pericardial capillaries were inflamed and generalised petechiation of the pericardium with haemorrhage on the internal surface of left ventricular wall was observed. Gross findings of the non-trial lamb included tracheal hyperaemia, petechiation of diaphragm, sub-capsular renal petechiation, fibrinous pericarditis, and liquid small intestinal content. No further diagnostic testing was performed.

Morbidity

No adverse events associated with vaccine administration were recorded. No clinical respiratory disease was recorded during repeated assessments. Relatively low mean levels of lameness, signs of diarrhoea, ocular discharge/entropion and orf lesions were recorded (Table 4). No significant effect of ear or orf lesions on DLWG were found. Orf was not observed on farms 4 and 6, nor in lambs that had received orf vaccination at pasture turnout (farm 5). Whilst entire ram lambs had heavier liveweights than ewe lambs (p<0.001), no significant interaction was found between sex and clinical outcomes. Compared to unaffected peers, those with signs of lameness or entropion/ocular discharge or diarrhoea were lighter (p<0.001).

Table 4 Number (n) and percentage (%) of trial lambs observed with specific clinical signs (secondary outcome) at each visit period on six sheep flocks

Clinical outcome	Score	n (%) lambs observed across all farms with each outcome at each visit (n=6)						Total n (%) lambs assessments
		1	2	3	4	5	6	
Ocular lesion	No lesions observed	851 (94.6)	872 (99.4)	860 (97.6)	776 (95.2)	725 (98.1)	411 (99.8)	4513 (97.0)
	Discharge	25 (2.8)	11 (1.24)	20 (2.27)	27 (3.31)	10 (1.35)	1 (0.24)	94 (2.0)
	Entropion	24 (2.7)	4 (0.5)	1 (0.1)	12 (1.5)	4 (0.5)	0 (0)	45 (1.0)
Ear tag lesion	No lesions observed	898 (99.8)	849 (95.7)	867 (98.4)	811 (99.5)	736 (99.6)	411 (99.8)	4590 (98.7)
	Ripped or torn	0 (0)	2 (0.2)	6 (0.7)	4 (0.5)	2 (0.3)	1 (0.3)	15 (0.3)
	Infection or abscess	2 (0.2)	36 (4.1)	8 (0.9)	0 (0)	1 (0.1)	0 (0)	47 (1.0)
Diarrhoea	Absent	881 (97.9)	883 (99.6)	861 (97.7)	800 (98.2)	720 (97.4)	408 (99.0)	4571 (98.3)
	Present	19 (2.1)	4 (0.4)	20 (2.3)	15 (1.8)	19 (2.57)	4 (0.97)	81 (1.7)
Orf	Not observed	888 (98.7)	782 (88.2)	874 (99.2)	815 (100)	739 (100)	412 (100)	4521 (97.4)
	Active or healing lesion	12 (1.3)	104 (11.6)	7 (0.8)	0 (0)	0 (0)	0 (0)	123 (2.7)
Gait	Sound	899 (99.9)	887 (100)	880 (99.9)	808 (99.1)	723 (97.8)	399 (96.8)	4592 (98.7)
	Lame	1 (0.1)	0 (0)	1 (0.1)	7 (0.9)	16 (2.2)	13 (3.2)	38 (0.8)

Liveweight

Trial group liveweights at study entry (visit 1) were not significantly different and showed no evidence of systematic allocation bias (Table 5).

Table 5 Mean, standard deviation (SD) and range (min–max) in liveweight (kg) of unvaccinated, early and late vaccinated trial lambs at each of the six visits (V1 to V6) on six sheep flocks

Vaccination status	Statistic	Visit					
		V1	V2	V3	V4	V5	V6
Unvaccinated	n	300	300	299	276	249	135
	Mean liveweight	16.6	25.0	30.3	34.3	37.3	41.8
	SD	3.5	4.3	5.4	5.8	5.9	4.9
	Min–max	7.0–26.5	13–38	11.6–45.0	18.0–48.0	22.0–51.0	25.0–51.5
Early vaccination	n	300	298	293	269	250	143
	Mean liveweight	16.5	24.5	30.0	33.8	36.2	41.6
	SD	3.2	4.1	5.5	5.7	19.0–49.0	4.8
	Min–max	8.0–25.0	13–27	16.0–46.0	15.0–51.0	6.2	24.0–51.5
Late vaccination	n	300	289	289	269	240	134
	Mean liveweight	16.4	24.9	30.2	34.5	36.8	41.8
	SD	3.2	4.0	5.3	5.4	6.1	4.7
	Min–max	8.4–26.0	14.2–37.0	17.6–44.0	20.0–48.0	22.4–49.0	28.0–54.5

Mean liveweight varied between farms (Table 5). Mean finishing weight (farms 1 to 5) was 44.9 kg. The trial ended before lambs on farm 6 reached finishing weights. Mean DLWG generally decreased over time (W1 to W5), particularly after weaning and prior to visit 4 (Table 6). Mean time from study entry to finishing was 116 days (95% CI 112–120 days); i.e. on average lambs reached slaughter weight at 6 months old. Farm identity and visit period had a significant effect on liveweight (p<0.001). When the full dataset and dataset excluding farms 1 and 6 were examined, and when farm identity, visit number, sex and animal identity were accounted for, no significant effect of vaccination on liveweight or DLWG was found. Furthermore, there was no significant difference in DLWG between unvaccinated and early vaccinated lambs up to weaning.

Table 6 Mean, standard deviation (SD) and range (min–max) in daily liveweight gain (DLWG) for consecutive weight gained in the time between visits 1 to 2 (W1), visits 2 to 3 (W2), visits 3 to 4 (W3), visits 4 to 5 (W5), visits 5 to 6 (W5) and overall DLWG (W0) of trial lambs on six sheep farms

Weight Gain	Statistic	Farm ID					
		Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6
W1	Mean DLWG (g/day)	312.4	372.2	333.4	264.4	252.9	192.0
	SD	54.4	73.0	78.0	58.0	63.5	57.7
	min–max	162.2–459.5	189.7–569.0	71.43–535.7	115.4–423.1	-40.0–393.3	-86.2–362.1
W2	Mean DLWG (g/day)	316.8	205.1	242.9	162.2	161.42	85.6
	SD	69.9	69.8	78.2	70.74	85.9	49.5
	min–max	74.1–555.6	55.56–388.9	-35.7–607.1	-112.9–451.6	-214.3–592.9	76.9–307.7
W3	Mean DLWG (g/day)	278.6	270.10	212.2	153.9	119.3	64.7
	SD	99.0	76.7	75.1	85.1	61.6	62.5
	min–max	0–466.7	71.4–464.3	-111.11–388.9	-111.1–463.0	-73.17–419.5	-132.4–294.1
W4	Mean DLWG (g/day)	182.9	167.4	71.7	190.8	66.6	28.8
	SD	93.4	78.7	64.0	72.3	66.2	82.0
	min–max	40.81–346.2	-42.86–382.4	-68.97–400.0	0–341.5	-137.9–324.1	-177.4–241.9
W5	Mean DLWG (g/day)	[a]	184.0	88.5	89.6	150.4	[a]
	SD	[a]	73.25	68.5	101.08	54.14	[a]
	min–max	[a]	-42.9–328.6	-309.5–312.5	-166.7–344.8	-5.9–270.6	[a]
W0	Mean DLWG (g/day)	309.6	248.6	202.8	181.9	147.8	92.1
	SD	50.2	45.36	69.23	35.49	33.1	27.0
	min–max	155.4–414.1	159.1–357.1	71.4–482.1	90.9–307.7	3.5–282.8	17.2–224.1

[a] Insufficient data for analysis: most lambs finished before visit 6 (Farm 1) or the trial had already ended (Farm 6)

DISCUSSION

To the authors' knowledge, following a literature search on the subject, no previous studies assessing field performance of multivalent Clostridial *spp.* and *Pasteurella* vaccines on British sheep flocks have been reported. In contrast to the medical sector, veterinary vaccine field trials are often considered inferior to challenge trials because of the lack of standardised conditions (Knight-Jones et al., 2014). However, vaccines need to perform under farming conditions where variation in individual animal immunity, pathogen exposure (Knight-Jones et al., 2014) and environmental and husbandry factors influence disease aetiology (EMEA, 2001). Well-designed and well-conducted field trials are necessary for informing evidence-based management of livestock (Sanderson, 2006; Dean et al., 2015).

Trial design and conduct
The Veterinary All Trials initiative (www.vetalltrials.org) supports the open publication of all trial outcomes and veterinary clinical trial results in order to prevent duplication of unreported studies, improve animal welfare

and improve the quality of studies used to inform evidence-based decision making (Dean et al., 2015). This article serves to highlight trial results, and discuss planned study design and conduct considerations together with actual field experiences to inform and improve further studies. A 'gold standard' randomised controlled trial (RCT) was not feasible due to limited personnel and financial resources. Instead, we used the REFLECT guidelines (Sargeant et al., 2010) to inform field trial design, conduct and reporting of study results. Blinding of the veterinarian, researcher and producers was performed as far as possible. Due to the nature of veterinary practice-based research and the need for producer involvement in animal handling, there were some practical limitations. Participants and researchers were not blinded to the treatment administered (i.e. the type or brand of vaccine). Producers presented a sample of twin lambs considered representative of the flock. Systematic randomisation was performed to reduce introduction of sample selection bias. To reduce observer scoring and recall biases, lambs were assessed by the same veterinarian who was blinded to data recorded at previous trial visits. To minimise potential measurement errors, farm weighing systems were calibrated at each visit, before use. To minimise the effect of competitive maternally-derived antibodies (MSD Animal Health, 2015), all early vaccine lambs received their first primary vaccine dose aged 6 weeks or above.

Expected ear tag losses in commercial systems were the reason for having both individual and group identification methods. The high level of tag losses (26.7%) on farm 3 may reflect the tagging technique, that a different brand of tag was used, or fencing type. Fleece colour was sufficient for group identification, and colour codes were withheld from the veterinarian and producers. Since fleece marks were clearly visible, trial participants were not fully blinded. Up to weaning, all trial lambs on each farm were managed as a single group, and post-weaning were managed as a group of trial ewe lambs and a group of trial ram lambs. There was no evidence that lambs of different vaccination status were deliberately managed in a different way due to incomplete blinding. Limited resources necessitated data entry by the researcher. Modelling, however, was supported by a statistician (PJC) blinded to vaccine status.

Generalisability

Trial farms were considered representative of farms and production types within the practice area and represented a convenience sample. Therefore, mortality, liveweight and DLWG data must be interpreted in light of the approximate age and timing of visits. Such data cannot be considered representative of all commercial English sheep flocks nor should it be used for benchmarking of flocks of a similar production type.

Mortalities and necropsy findings

The study identified a lower level of mortality in fully vaccinated lambs, however vaccination alone did not prevent lamb mortalities. 13.3% (n=4) of trial mortality cases occurred in lambs classed as fully vaccinated, and these mostly arose in the early vaccination group. Aside from Farm 1, the majority of grazing lamb losses occurred in the week immediately after weaning or in the month following weaning. This timing appears to coincide with the period 12–16 weeks after ewe vaccination when colostral antibodies to clostridal *spp*. are purported to wain in lambs which have received adequate passive transfer of immunity from correctly vaccinated ewes (MSD Animal Health, 2015). However, due to the very low level of lambs presented for necropsy investigations, the causes of death in both vaccinated and unvaccinated trial lambs were not diagnosed.

Mortality data capture and necropsy were not undertaken during the field *Pasteurella spp*. vaccine trial by Goodwin-Ray et al. (2008) due to the expected logistical difficulties for accurately monitoring on New Zealand flocks. Given the smaller English trial flock sizes in this study, accurate producer-recording of mortalities was considered feasible. All flocks were within a 15 mile radius of the veterinary practice, and participating producers were offered free-of-charge necropsies. Despite this, only one study flock (farm 1) submitted lambs (one trial and one non-trial lamb) for necropsy during the study. The low level of necropsy uptake was disappointing. Whilst producers recognised the value of necropsy for informing management decisions, a commonly reported issue was the extensive degree of scavenging and carcase decomposition when lambs were found dead, which concurs with the concerns of researchers in the earlier New Zealand study. The

present trial highlights the real logistical and practical issues with monitoring mortalities in grazing lambs, even when recruiting an engaged and committed group of producers to veterinary practice-based research.

A total of 30 mortalities were registered but the lack of individual animal identity and poor recovery rate of ear tags from decomposed or scavenged carcases, and the loss of ear tags between visits, particularly on farm 3, initially presented some challenges for data analysis. However, since vaccine status was colour coded, applying the assumption that lambs missing for at least two visits and not slaughtered had died, these elements facilitated confirmation of the vaccination status of nearly all mortality cases. On farm 1, where no multivalent *Clostridia spp*. or *Pasteurella* preventive ewe vaccination was historically undertaken, all trial lamb deaths occurred pre-weaning in either unvaccinated lambs, or in partially vaccinated lambs prior to completion of their primary vaccination course. Sudden deaths of prime lambs that were near to reaching slaughter weight were reported on farm 1, but did not occur in the trial lamb group. Necropsies on farm 1 revealed gross signs suggestive of pasteurellosis, and enterotoxaemia due to *Clostridium perfringens* D toxin (colloquially: 'pulpy kidney'). Further investigations, including bacteriology, brain histology and epsilon toxin testing, required for definitive diagnosis of clostridial enterotoxaemia (Uzal et al., 2004) were not conducted due to limited study resources. Therefore, these diseases and other causes of septicaemia could not be excluded.

Lambs on farm 1 may have been at a higher risk of clostridial disease and pasteurellosis prior to weaning since they were offered creep feeding throughout the trial period, whereas on farms 2–4 – where creep feeding was offered post-weaning – the change in diet and stress associated with weaning may explain the higher number of lambs that died post-weaning. Evidence from this study and earlier clinical reports (Lewis, 2007) support the value of preventive ewe vaccination as a means of reducing lamb mortality on farm 1 associated with preventable disease. Lamb losses or clinical signs (respiratory disease) associated with *Pasteurella spp*. were not definitively diagnosed in any of the trial groups, which may reflect the very low level of necropsy submissions and/or a low level of disease challenge during the study year. Therefore, insufficient data were captured to give useful diagnostic information, specifically information regarding the prevention of pastuerellosis, which had been the background for this practice-based trial. Future researchers might recruit housed lambs to improve capture of mortality cases for diagnostic investigations, which was not the approach used here since the aim was to capture data generalisable to pasture-reared lambs.

Lamb growth rates
Significant between-farm and visit-period differences in growth rate (DLWG) were found, which likely reflect differing management practices (e.g. creep feeding of lambs). Across all farms and irrespective of vaccination group, the highest mean DLWG (277.4 g/day) was recorded early on, between visits 1 and 2, when lambs were between 6 and 12 weeks old, and likely reflects peak milk production of ewes. With the exception of two flocks (farms 5 and 6), the mean DLWG over the study period was above 150 g/day. Highest mean DLWG was recorded on farm 1 and may be explained in part by that farm's earlier lambing period, which may be associated with reduced parasite and other disease challenges and, most likely, early concentrate feeding of lambs prior to weaning. By comparison, lambs born later in the season, grazed in severely disadvantaged areas (SDA) (e.g. on farm 5 and 6) and that did not receive supplementary concentrate feeding had a lower mean DLWG.

Reduced lamb growth rates can have considerable impacts on flock performance associated with delays in finishing time to slaughter, missing the peak in market prices, submission of less-uniform lambs to slaughter, increased numbers of lambs kept as stores, and the subsequent effects on ewe lamb fertility and performance (Gascoigne and Lovatt, 2015). In this study, mean DLWG reduced over the trial period on all farms and decreased considerably post-weaning, prior to visit 4 (98.9 g/day), but improved by visit 5 (122.6 g/day). Weaning is a stressful event, with changes to lamb nutrition, social stability and other factors. Reduced immunity and increased host susceptibility to infectious and parasitic disease may explain reduced DLWG observed at visit 4. However, disease and parasite monitoring were not performed systematically across these flocks. Further investigations, for example including DLWG recording of the wider flock, close monitoring of

parasite burdens in grazing lambs, diagnosing the reasons behind mortalities, and understanding grazing management and disease control practices may identify multiple factors associated with reduced growth rates around weaning time.

Vaccination was not associated with a significant difference in lamb growth rate. Sample size calculations were based on *a priori* assumptions on mortality differences between vaccinated and unvaccinated lambs. Therefore, the lack of statistical significance in analysis of the secondary outcome (DLWG) may reflect a Type I statistical error (inadequate sample size to identify a true effect) or a true effect. However, significantly reduced growth rates in finishing lambs with severe lung lesions associated with *M. haemolytica* or *P. multocida* have been identified in a trial with 259 lambs (Daniel et al., 2006), so the sample size of 900 lambs presented here may have been sufficient to detect a meaningful effect. A lack of significant differences in DLWG was also found in a New Zealand study that tested a multivalent *Pasteurella* vaccine (Ovipast P Plus, MSD Animal Health, 2015) on 9174 lambs from six commercial flocks (Goodwin-Ray et al., 2008). Testing of the same *Pasteurella spp.*-specific vaccine on English sheep flocks was considered but given the additional requirements for handling and vaccination costs, and in light of the current vaccine routines and producer identified knowledge gap, this trial was instead designed to examine an alteration in timing of commonly practiced (post-weaning) vaccination of lambs with a multivalent *clostridia* and *Pasteurella* vaccine.

Disease incidence
An accepted a priori risk was that insufficient disease exposure to detect significant differences in study outcomes might occur. Herd immunity (i.e. protection afforded from vaccinated animals within the same management group) can affect the ability of studies to detect meaningful between-group differences (Dohoo et al., 2009). Therefore, flocks that were not already vaccinating lambs against clostridial disease or pasteurellosis, and those with confirmed/suspected disease, were recruited. Furthermore, unvaccinated lambs were left within each trial group with the aim of maintaining some disease challenge. Remaining lambs in each flock were left unvaccinated as per standard farm practice. Despite this, routine preventive breeding animal vaccination on five farms likely reduced disease challenge. Following primary vaccination and pre-lambing boosters, datasheets suggest that the vaccine used offers lambs 12 to 16 weeks of colostral-derived antibody protection against some clostridial diseases; lamb dysentery, pulpy kidney and tetanus (MSD Animal Health, 2015). Based on the datasheet advice of unproven, but reported, duration of up to 4 weeks of *Pasteurella*/*Mannheimia* passive immunity derived from colostrum, it was assumed that pasteurellosis challenge was not markedly reduced for trial lambs. All producers participating in the trial perceived a lower level of respiratory disease outbreaks in lambs during 2013. Lack of recorded clinical respiratory signs may support this view of low disease exposure, although clinical observation is not a sensitive tool. Transthoracic ultrasonography is a more sensitive means of diagnosing ovine respiratory disease (Scott and Sargison, 2010), but was not applied here due to time and financial constraints. It is not possible to validate producer perceptions and trial findings with regional veterinary laboratory diagnostic data (supplementary material) due to the very low level of diagnoses, and unknown disease and vaccination status of submitting flocks. Overall, there is no strong evidence that suggests 2013 was an unusually low year for pasteurellosis in the region.

Orf cases peaked (11%) at visit 2 and declined considerably 1 month later. Reduced weight gain for up to 5 weeks after clinical orf lesions has previously been reported in three-week-old lambs (Lovatt et al., 2012). However, Lovatt et al. (2012) found no significant growth rate differences between orf affected and unaffected lambs aged 7 weeks and over, which concurs with our findings in lambs aged 6 weeks and over. Lameness (0.9%) and ocular conditions (3.3%) were lower than those previously reported (2.1% and 6.6%, respectively) in younger lambs (Phythian et al., 2013), which may reflect variation in sample age, pathogen exposure, management practices, and, potentially, greater veterinary involvement. Gait was assessed as lambs moved through handling areas, most frequently mobile systems, at pasture. Thus, lameness levels may represent only the most severe cases, and might underestimate true prevalence. Lame lambs, or those with entropion/ocular discharge or diarrhoea scour were lighter than unaffected peers. This is the first report providing evidence of a significant impact of ocular lesions (including discharge and entropion) on the growth rate of lambs aged 6

weeks old and above, and highlights the need for early intervention in affected lambs to reduce the animal welfare and production impacts associated with these conditions.

Future studies

A single group of trial lambs was not maintained for both animal welfare and practical reasons. On all farms, male and female trial lambs were separated at weaning and remained in a same-sex group of trial lambs. Since all farms managed all trial lambs in this way and the statistical methods were capable of taking account of individual farm differences, this effect was considered to be limited. Trial timing was based on peak lambing dates, logistical considerations, and assumed time to slaughter. One farm unexpectedly dropped out shortly before study commencement, and, as a consequence, a later lambing flock was recruited that did not sell lambs to slaughter before the trial ended (farm 6). Another flock (farm 1) had sold almost all lambs by the time the secondary course of the primary 'late' group vaccination was due. However, excluding farms 1 and 6 from the dataset made no significant difference to study findings. Whilst a larger sample than suggested by sample size estimates had been included to limit the impact of unpredicted issues (e.g. drop-out or unexpected disease), selecting farms with closer lambing dates, more similar management practices, as well as recruiting a much larger sample, and considering alternative design (e.g. a clustered random trial), could be worthwhile for future trials on pasture-managed sheep. Visits were not extended beyond 6 months due to flock management and trial financial resources. Future studies might consider only enrolling breeding lambs to facilitate longer-term evaluation of post-weaning vaccination. Although, findings of such studies may not be generalisable to commercial flocks rearing lambs for slaughter.

Conclusions

Overall, there was a clear trend in reduced mortality rates in grazing lambs that received a multivalent *clostridia* and *Pasteurella spp*. vaccine course, compared to unvaccinated lambs. No significant effects of pre- or post-vaccination on lamb growth rates were found. On one trial farm that did not undertake preventive clostridial and *Pasteurella* breeding animal vaccination, there was strong clinical and gross pathological suspicion of *Pasteurella* and clostridial disease in two lambs, which highlighted the disease risks for unvaccinated sheep flocks. Carcase scavenging and decomposition resulted in a very low submission rate of lambs found dead at pasture. This lack of veterinary necropsy examination resulted in a loss of valuable data on the causes of mortality in grazing lambs, which are likely to vary between flocks and differ between years. Whilst there may not be per se a cost benefit for ewe or lamb vaccination every year, best practice recommendations for maintaining an annual vaccination regime are based on maintaining 'herd immunity'. A whole farm approach to veterinary flock health planning with specific risk assessment, cost-benefit analysis and farm-specific evidence from lamb mortality data, supported by necropsy and diagnostic testing, could help to inform the decision to implement a new regime, or to amend the timing of preventive vaccination programmes for lambs.

Implications

Overall, there was evidence to support veterinary advice to implement preventive ewe vaccination on one unvaccinated flock (farm 1). This study cannot provide evidence to indicate the benefit of vaccination in flocks experiencing severe disease outbreaks associated with *Pasteurella spp*. There were fewer mortalities in lambs that were vaccinated prior to weaning ('early' vaccination group). However, this trial cannot provide evidence for a 'one-size fits all' recommendation for implementing pre-weaning vaccination of lambs in flocks that already adhere to best practice preventive ewe vaccination.

SUPPLEMENTARY MATERIALS

Table S1 Sheep Surveillance Dashboard 2012–2017 data for number of sheep in Devon submitted for necropsy to the regional Animal Plant and Health Agency (APHA) veterinary investigation centre in which *Pasteurella spp*. were diagnosed as cause of death

Year	n sheep with specific diagnoses		
	Systemic Pasteurellosis	*Pasteurella* (non-respiratory)	Pneumonia – *Pastuerella multocida*
2012	2	1	0
2013	1	2	1
2014	2	0	0
2015	1	0	0
2016	7	0	0
2017	5	4	1

Data extracted directly from the publicly available Sheep Disease Surveillance Dashboard:
https://public.tableau.com/profile/siu.apha#!/vizhome/SheepDashboard_/Overview
N.B Denominator data on total number of sheep submitted for necropsy over these periods were not provided.

CONFLICT OF INTEREST

REFERENCES

1. AHPA. Animal Health and Plant Agency Sheep Disease Surveillance Dashboard.

2. Al Saif, N. & Brazier, J.S. 1996. The distribution of *clostridium difficile* in the environment of South Wales. *Journal of Medical Microbiology*. 45: 2, 133–137. DOI: http://dx.doi.org/10.1099/00222615-45-2-133

3. Brown, K.K., Parizek, R.E. & Stewart, R.C. 1976. Prevention of clostridial disease in cattle and sheep by vaccination with a multivalent bacterin-toxoid. *Veterinary Medicine Small Animal Clinics*. 71: 12, 1717–1722.

4. Cassirer, E.F., Rudolph, K.M., Fowler, P., Coggins, V.L., Hunter, D.L. & Miller, M.W. 2001. Evaluation of ewe vaccination as a tool for increasing bighorn lamb survival following pasteurellosis epizootics. *Journal of Wildlife Diseases*. 37: 1, 49–57. DOI: http://dx.doi.org/10.7589/0090-3558-37.1.49

5. Chandrasekaran, S., Hizat K., Saad, Z, Johara, M.Y. & Yeap, P.C. 1991. Evaluation of combined *Pasteurella* vaccines in control of sheep pneumonia. *British Veterinary Journal*. 147: 5, 437–443. DOI: http://dx.doi.org/10.1016/0007-1935(91)90086-3

6. Daniel, J.A., Held, J.E., Brake, D.G., Wulf, D.M. & Epperson, W.B. 2006. Evaluation of the prevalence and onset of lung lesions and their impact on growth of lambs. *American Journal of Veterinary Research*. 67: 5, 890–894. DOI: http://dx.doi.org/10.2460/ajvr.67.5.890

7. Dean, R., Royle, N., Boulton, C., Turner, S., Mckenzie, B., O'Connor, A., Budsberg, S., Pion, P., Lambert, A., Jarvis, S. & Reynolds, S. 2015. Veterinary All Trials Initiative. *Veterinary Record*. 177: 5, 131–132. DOI: http://dx.doi.org/10.1136/vr.h4107

8. Donachie, W. 2007. Pasteurellosis. In Diseases of Sheep, Fourth edition. Ed. Aitken, I.D. Blackwell Publishing, Oxford, United Kingdom, pp 224–231.

9. Dohoo, I., Martin, W. & Stryhn, H. 2009. Controlled Studies. In: Veterinary Epidemiologic Research, Second Edition. VER Inc, Charlottetown, Prince Edward Island, Canada, pp 213–237.

10. European Agency for the Evaluation of Medicinal Products (EMEA). 2001. Note for guidance. Field trials with veterinary vaccines.

11. Gascoigne, G. & Lovatt, F. 2015. Lamb growth rates and optimising production. *In Practice*. 37, 401–414. DOI: http://dx.doi.org/10.1136/inp.h4537

12. Gilmour, N.J., Martin, W.B., Sharp, J.M., Thompson, D.A. & Wells, P.W. 1979. The development of vaccines against pneumonic pasteurellosis in sheep. *Veterinary Record*. 104: 15, 266–269. DOI: http://dx.doi.org/10.1136/vr.104.1.15

13. Green, D.S., Green, M.J., Hillyer, M.H. & Morgan, K.L. 1987. Injection site reactions and antibody responses in sheep and goats after the use of multivalent clostridial vaccines. *Veterinary Record*. 120, 435–439. DOI: http://dx.doi.org/10.1136/vr.120.18.435

14. Goodwin-Ray, K.A., Stevenson, M.A. & Huer, C. 2008. Effect of vaccinating lambs against pneumonic pasteurellosis under New Zealand field conditions on their weight gain and pneumonic lung lesions at slaughter. *Veterinary Record*. 162, 9–11. DOI: http://dx.doi.org/10.1136/vr.162.1.9

15. Jones, G.E., Donachie, W., Sutherland, A.D., Knox, D.P. & Gilmour, J.S. 1989. Protection of lambs against experimental pasteurellosis by transfer of immune serum. *Veterinary Microbiology*. 20: 1, 59–71. DOI: http://dx.doi.org/10.1016/0378-1135(89)90007-2

16. Knight-Jones, T.J.D., Edmond, K., Gubbins, S. & Paton, D.J. 2014. Veterinary and human vaccine evaluation methods. Proceedings of the Royal Society. 281.
DOI: http://dx.doi.org/10.1098/rspb.2013.2839

17. Lewis, C.J. 2007. Clostridial diseases. In: Diseases of Sheep, Forth edition. Ed Aitken, I.D. Blackwell Publishing, Oxford, United Kingdom, pp 156–167.

18. Lovatt, F.M., Barker, W.J.W., Brown, D. & Spooner, R.K. 2012. Case-control study of orf in preweaned lambs and an assessment of the financial impact of the disease. *Veterinary Record*. 170, 673–677.
DOI: http://dx.doi.org/10.1136/vr.100646

19. Malone, F.E., McCullough, S.J., McLoughlin, M.F., Ball, H.J., O'Hagan, J. & Neill, S.D. 1988. Infectious agents in respiratory disease of housed, fattening lambs in Northern Ireland. *Veterinary Record*. 122, 203–207. DOI: http://dx.doi.org/10.1136/vr.122.9.203

20. MSD Animal Health. 2015. Datasheet for Heptavac-P Plus.

21. Phythian, C.J., Cripps, P.J., Michalopoulou, E., Jones, P.H., Grove-White, D., Clarkson, M.J., Winter, A.C., Stubbings, L.A. & Duncan, J.S. 2012. Reliability of indicators of sheep welfare assessed by a group observation method. *The Veterinary Journal*. 193: 1, 257–263.
DOI: https://doi.org/10.1016/j.tvjl.2011.12.006

22. Phythian, C.J., Toft, N., Cripps, P.J., Michalopoulou, E., Winter, A.C., Jones, P.H., Grove-White, D. & Duncan, J.S. 2013. Inter-observer agreement, diagnostic sensitivity and specificity of animal-based indicators of young lamb welfare. *Animal*. 7: 7, 1182–1190.
DOI: http://dx.doi.org/10.1017/S1751731113000487

23. RUMA. 2006. Responsible use of medicines in agriculture alliance guidelines. Responsible use of vaccines and vaccination in sheep production.

24. Sanderson, M.W. 2006. Designing and running clinical trials on farms. *Veterinary Clinics of North America: Food Animal Practice*. 22: 1, 103–123. DOI: http://dx.doi.org/10.1016/j.cvfa.2005.11.004

25. Sargeant, J.M., O'Connor, A.M., Gardner, I.A., Dickson, J.S., Torrence, M.E., Dohoo, I.R., Lefebvre, S.L., Morley, P.S., Ramirez, A. & Snedeker, K. 2010. The REFLECT statement: reporting guidelines for randomized controlled trials in livestock and food safety: explanation and elaboration. *Journal of Food Protection*. 73: 3, 579–603. DOI: http://dx.doi.org/10.4315/0362-028X-73.3.579

26. Scott, P.R. & Sargison, N.D. 2010. Ultrasonography as an adjunct to clinical examination in sheep. *Small Ruminant Research*. 92: 1-3, 108–109. DOI: http://dx.doi.org/10.1016/j.smallrumres.2010.04.011

27. Uzal, F.A., Kelly, W.R., Morris, W.E., Bermudez, J. & Baisón, M. 2004. The pathology of peracute experimental *Clostridium perfringens* type D enterotoxemia in sheep. *Journal of Veterinary Diagnostic Investigation*. 16: 5, 403–411. DOI: http://dx.doi.org/10.1177/104063870401600506

28. Uzal, F.A. 2004. Diagnosis of *Clostridium perfringens* intestinal infections in sheep and goats. *Anaerobe*. 10: 2, 135–143. DOI: http://dx.doi.org/10.1016/j.anaerobe.2003.08.005

29. Wells, P.W., Evans, H.B., Burrells, C., Sharp, J.M., Gilmour, N.J., Thompson, D.A. & Rushton, B. 1979. Inability of passively acquired antibody to protect lambs against experimental pasteurellosis. *Infection and Immunity*. 26: 1, 25–29. DOI: http://dx.doi.org/10.1128/IAI.26.1.25-29.1979

30. Wells, P.W., Robinson, J.T., Gilmour, N.J., Donachie, W. & Sharp, J.M. 1984. Development of a combined clostridial and *Pastuerella haemolytica* vaccine for sheep. *Veterinary Record*. 114, 266–269. DOI: http://dx.doi.org/10.1136/vr.114.11.266

31. Zheng, T., Gupta, S.K., McCarthy, A.R., Moffat, J. & Buddle, B.M. 2015. Cross-protection study of a *Mannheimia haemolytica* serotype 1 vaccine against acute pasteurellosis in lambs induced by a serotype 2 strain. *Veterinary Microbiology*. 177: 3-4, 386–393. DOI: http://dx.doi.org/10.1016/j.vetmic.2015.02.019

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