Summary Report for DARD Research Challenge Fund Project: Serological survey to determine prevalence of Northern Ireland suckler and dairy herds with evidence of current or recent infection with Bovine Viral Diarrhoea virus

DARD Research Challenge Fund Project
Co-funded by Agrisearch

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1 Summary

Bovine Viral Diarrhoea (BVD) is an endemic disease of cattle that causes significant losses to beef and dairy herds worldwide. The goal of this study was to estimate the prevalence of suckler and dairy herds in Northern Ireland (NI) with serological evidence of current or recent circulation of bovine viral diarrhoea virus (BVDV). Dairy and suckler herds undergoing a routine brucellosis herd screen between April 2011 and June 2012 were randomly selected and tested using a young stock check test. A minimum of 5 and a maximum of 10 homebred young animals (12-24 months of age) per herd were tested for evidence of antibodies to BVDV p80 protein. Presence of antibodies in this group indicates that they have been in contact with BVDV, typically from an animal persistently infected with the virus, and is strong evidence of current or recent infection in that herd. On the other hand, the presence of antibodies in one or more animals indicates that virus has been circulating in the herd during the lifetime of the animals sampled, and therefore that the herd is currently (or has recently been) infected with BVDV. A total of 5,161 animals in 589 herds and were sampled and tested. A total of 5,163 animals from 589 herds were sampled and tested. In 34% of them (202 herds) all animal tested were seronegative; in 14% of the herds (83 herds) all animals were seropositive. In the remaining herds, one animal or more were positive for BVDV antibodies. 66% of the herds showed some degree of exposure to the virus (true prevalence 67.37% (62-72.5%)). The impact of the eradication of BVDV from NI dairy and suckler herds on green house gas emissions was evaluated. It was estimated that it would result in CO_2e savings equivalent to £5.38 million/year.

2 Introduction

Bovine viral diarrhoea virus (BVDV) is an economically important pathogen present in Northern Ireland dairy and beef herds. The disease is widespread affecting cattle in most countries around the world. Losses may be associated with a wide range of reproductive effects, including conception failure and abortion, suppression of the immune system in calves resulting in increased levels of pneumonia and diarrhoea, reduced milk yields, increased somatic cell counts and death of animals from mucosal disease, characterised by lameness, scour and ulceration of the gastrointestinal tract. Calves infected in the first trimester of pregnancy may develop immunotolerance against the infecting strain of BVDV and are later born persistently infected (PI) with the virus (Brownlie, J, Clarke, M C, Howard 1989). These calves continuously shed large amounts of the virus and are the main source of infection to the rest of the herd, playing a significant role in the epidemiology of

BVDV. Therefore, herds with PI animals, often have a high prevalence of seropositive animals (Houe et al. 1995, Van Campen et al. 1998).

In the UK and Ireland the 'do nothing' approach to BVDV has been common, with only voluntary schemes, such as the CHeCS licensed schemes, in place. However, the situation is developing rapidly. After a voluntary phase, the Scottish Government commenced the compulsory phase of the Scottish BVD eradication programme in December 2011. The eradication of this disease alone is being estimated to contribute 3% of the required green house gas (GHG) savings from Scottish agriculture.

In the Republic of Ireland (RoI) there has been ongoing activity on BVDV control, which has been led by Animal Health Ireland (www.animalhealthireland.ie). A voluntary phase of a BVD eradication programme started in January 2012 which has been followed by a compulsory phase in January 2013. This is a programme based on the method taken by Switzerland, where a new approach for the eradication of BVD has been used (Presi & Heim 2010). The scheme is based on the direct antigen testing of calves for BVD virus by using tissue samples collected with ear tags. It consists of three years of tissue tag testing of all calves followed by 3 years of monitoring.

At the time of this study (2011-12), in Northern Ireland only voluntary programmes existed. These programmes are based on CHeCS licensed schemes and offer testing regimes and guidance to attain and maintain BVD freedom and subsequent certification. However, progress of BVD eradication schemes in the RoI and Scotland has stimulated industry-led discussions on the feasibility of a programme for NI. In order to inform this debate and to help estimate the cost, logistics and timescales of a BVDV eradication programme for Northern Ireland, it was necessary to investigate the prevalence of dairy and suckler herds which currently are, or recently have had, active infection with BVDV. Such base-line knowledge defined the current scale of the problem and has aided in development of strategies for BVD control. A voluntary BVD eradication programme for NI started on January 2013.

3 Objectives

- To generate a statistically valid figure for the percentage of beef and dairy herds which currently have, or recently have had, active infection with BVDV.
- Use this figure to inform discussion and decisions on the feasibility, costs and timescales for a NI BVDV eradication programme.
- Estimate the GHG savings that such a programme could deliver in terms of the overall mitigation target for agriculture.

4 Materials and methods

4.1 Study design

A cross-sectional study targeting young stock from randomly selected dairy and suckler herds located in Northern Ireland was carried out. The target was sampling 350 dairy and 350 suckler herds between December 2010 and December 2011.

A sampling frame for dairy and beef herds was drawn from a national computerised database maintained by the Department of Agriculture and Rural Development (DARD). Only dairy herds with 20 or more dairy cows (n = 2,860) and beef suckler herds with 10 or more female breeding cattle (n = 7,984) were included in the sampling frame. Herds under brucellosis restriction were excluded from the sampling frame. A random sample of dairy herds and beef suckler herds (assuming a 70% and 60% participation rate, respectively) was drawn. Each selected herd was marked on the national database to indicate that the herd keeper was to be asked to participate in the survey. Agreement or otherwise to take part in the survey was recorded on the database. Due to a delay in the starting of the survey, a further sample was drawn to ensure that an adequate number of herds participated. Dairy herds with a minimum of 20 females (cows over 24 months) and suckler herds with a minimum of 10 breeding animals (female breeding cattle over 24 months) were eligible to be included in the study. In addition, herds had to have a minimum of 5 homebred animals between 12 and 24 months old.

DARD Animal Health and Welfare Inspectors (AHWIs) routinely visit farms to collect the brucellosis blood samples by venepuncture. When a herd was marked as a participant on the database, AHWIs took the study authorisation form and leaflet to the farm. If the herd owner agreed to participate, the signed authorisation form was sent with the samples to the brucellosis laboratory by courier with the contact telephone number and best time of the day to contact.

Herd testing was carried out using a modification of the “check test” approach described in the CHeCS Technical Document (www.checs.co.uk). This tests homebred young stock (9-18 months of age) for evidence of antibodies to BVDV. Due to the brucellosis sampling being targeted to animals over 12 months, it was necessary to increase the age range of the testing population for this study to animals aged between 12 and 24 months. A maximum of 10 animals in this age range were tested for BVDV antibodies per herd. In herds where there were only five eligible animals and were all seronegative to BVDV, one of them was randomly tested for BVD virus by antigen capture enzyme linked immunosorbent assay (ACE) following CHeCS rules advice. The antigen testing is carried out in case all the animals sampled are persistently infected with BVD. Although not highly probable, it is possible that a full generation of PI animals are born within a herd.

Due to data protection issues, the start of the herd sampling was delayed until April 2011 with the exception of 4 pilot herds sampled in February 2011. Brucellosis testing is very seasonable, with a marked decrease in the summer period, when a lot of the cattle is grazing. The completion date for the sample phase was initially April 2012 but, as the targeted number of herds had not been recruited, the sampling period was extended with a small number of herds added in May and June. After reviewing the number of herds in the study, at this point it was concluded that there were enough to have a representative sample of Northern Irish herds and the sampling was brought to an end.

A total of 5163 blood samples from 589 herds were tested.

4.2 Serological tests

A commercial enzyme-linked immunosorbent assay (ELISA) was used to test individual samples (LSI Vet BVD/BD p80 blocking one step, Laboratoire Service International) for antibodies against BVDV as per the manufacturer's instructions. Results are expressed in % inhibition (%inh) which is calculated by subtracting the optical density (OD) of the sample minus the negative control OD, dividing it by the negative control OD and multiplying by 100.

For individual serum samples, a result is considered negative if the %inh is < 50, low positive if between 50 and 80 %inh and high positive when ≥ 80 %inh. The assay has a sensitivity of 96.9% and a specificity of 97.8% (manufacture's validation report).

The pooled serum sample from each herd was also tested for antibody levels with the same commercial kit following the protocol for pooled serum for diagnostic application in pools of 2 to 10 samples. According to this, pool samples and controls were diluted 1/2 in sample dilution buffer (as opposed to testing individual samples diluted 1/10). According to manufacturer's instructions, if the result obtained was < 30 %inh, the pool status is 'non infected'. If the result obtained was ≥ 30 %inh, the pool status is 'recently infected or infected'. Individual and pool results were reported to the veterinary practitioners indicated by the herd owners and directly to the herd owners.

4.3 Virological tests

A commercially available ACE (Herd Check BVDV antigen/serum plus, IDEXX Laboratories) for the detection of E^{rns} virus protein was used to test serum according to the manufacturer's instructions.

The presence or absence of BVDV antigen was determined by calculating the corrected optical density value (COD) or S-N. The optical density values (ODs) were measured at 450 nm, and the CODs of samples and positive control then calculated by subtracting the mean OD for the negative controls from the obtained OD (COD (S-N)= $OD^{obtained}$ - mean $OD^{negative\ controls}$). Serum samples with S-N ≤0.3 were considered negative and S-N > 0.30 were considered positive.

4.4 Data analysis

Obtained apparent herd prevalence estimates were converted in true prevalence estimates by taking account of the sensitivity and specificity of the used test. For the individual antibody ELISA, the values of 96.9% sensitivity and a specificity of 97.8% were assumed (manufacture's validation report).

5 Brief Herd description

Herds located in all of the six Northern Irish Counties were included in the study. A higher proportion of herds from County Down and Tyrone and a lower from Armagh were sampled (Table 1). The distribution of herds sampled by County matched that found on APHIS descriptive statistics for 2011 (**Error! Reference source not found.**). Between 3-4% of herds within each County were sampled in the study and at a Northern Ireland level, 3% of the total herds were tested (Table 1).

Table 1: Number of herds tested by County

County	Total	% study herds	Expected %	Number of herds 2011	% tested
Antrim	85	14%	13%	2455	3%
Armagh	58	10%	8%	1545	4%
Down	152	26%	22%	4149	4%
Fermanagh	80	14%	15%	2871	3%
Londonderry	98	17%	15%	2768	4%
Tyrone	116	20%	26%	4833	3%
Total	**589**			**18621**	**3%**

Herds were allocated to a herd type according to the proportion of breeding cows. Those with ≤80% of beef suckler cows were considered suckler herds and those with ≤80% of dairy cows, dairy. 225 herds (38%) were suckler herds, 337 (57%) were dairy and 27 (5%) were dual purpose herds (Table 2). When herd owners were asked what type of herd they managed at the time of filling the questionnaire, a slight different percentage was obtained for those considered to be of dairy and dual purpose. DARD data for 2011 shows that there is a higher proportion of suckler or beef herds than dairy or dual purpose (breeding herds). 13% of dairy herds were tested in the study whilst only 1.6% of the suckler and 1.4% of dual herds were included.

Table 2: number and percentage of herds by type according to APHIS and herd owner

	In study		2011 data		Tested
	Number	Percentage	Number	Percentage	
Suckler	225	38%	14052	75.5%	1.6%
Dairy	337	57%	2620	14.1%	13%
Dual	27	5%	1949	10.5%	1.4%

The herds in the study had between 8 and 800 female animals over 2 years old. Due to the inclusion criteria by which dairy herds had to have a minimum of 20 breeding animals and suckler herds a minimum of 10, very few herds in the study have lower or equal to 10 female breeding animals (Table 3).

Table 3: distribution of herds by herd size

	Female animals >2 years	
	Number	Percentage
≤ 10	6	1%
11 to 25	64	11%
26-50	149	25%
51-100	193	33%
101-150	93	16%
151-259	69	12%
>250	15	3%
	589	

6 Results

6.1 Serological results

The distribution of herds by the proportion of seropositive animals when samples were tested individually is illustrated in Figure 1. In 202 herds (34%) all the animals tested were seronegative and in 83 (14%) all the animals were seropositive. In 185 herds (32%) antibody was detected in between 10 and 50% of the tested animals and in 119 herds (20%) between 50 and 90% of the tested animals were seropositive.

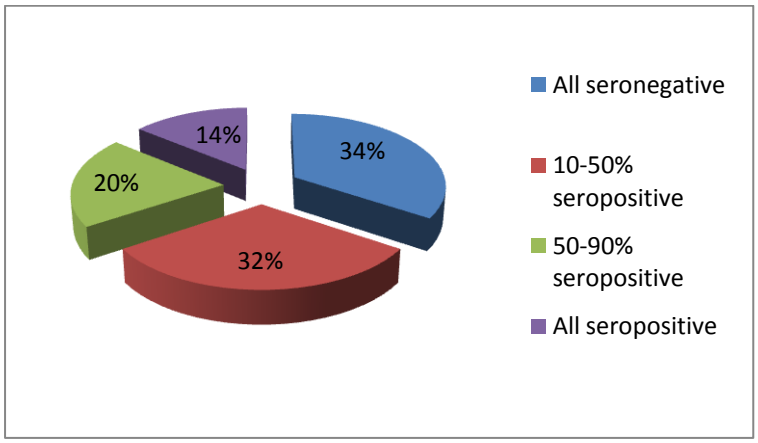

Figure 1: distribution of herds with no seropositive, all seropositive, 10-50% seropositive and >50% seropositive

A total of 5,161 serum samples were tested for antibodies to BVDV p80 protein. 1,184 (23%) were high positive, 656 (13%) were low positive and 3,321 (64%) negative. The distribution of individual results can be seen in Figure 2 and the frequency of distribution of all the individual results obtained in %inh in Figure 3.

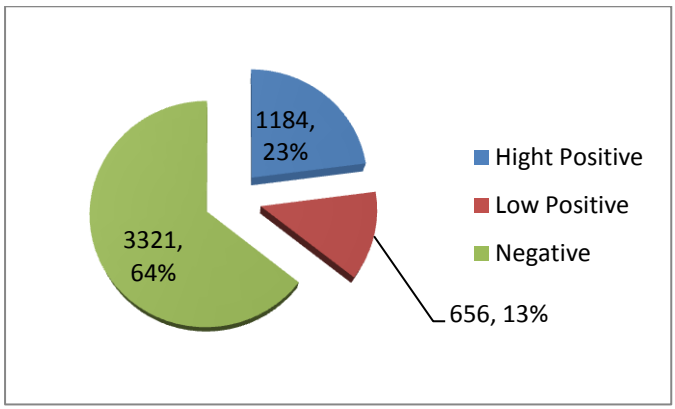

Figure 2: result of individual samples tested for BVDV p80 antibody (LSI Di)

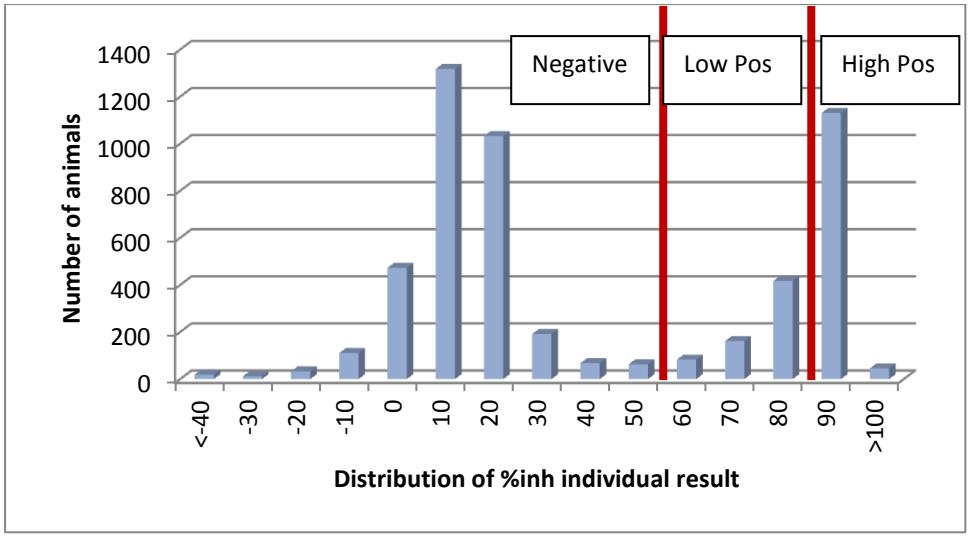

Figure 3: distribution of %inh results obtained on the testing of individual serum sample with LSI Di. Negative < 50 %inh, low positive 50-80 %inh, high positive > 80 %inh

Serological BVDV true prevalence was estimated at herd level and stratified by herd type based on the proportions of dairy and suckler cows (Table 4). There were no significant differences between herd types.

Table 4: serological BVDV true herd prevalence and number of seropositive animals per herd type. Apparent prevalence estimates were based on a generalized estimating equation and converted into true prevalence estimates

	Seropositive herds	Herd seroprevalence	Seropositive animals
Dairy	218/337	65.98% (58.93-73.02)	1100/3158
Suckler	151/225	68.54% (59.58-77.49)	640/1766
Dual	18/27	68.06% (42.38-93.73)	101/237
Total	**387/589**	**67.37% (62-72.5)**	**1841/5161**

Seropositive herds: herd with at least one seropositive animal

Of the 587 pools tested, 191 pools (33%) gave a %inh <30 considered to be a negative result according to the manufacturer's interpretation. The remaining 396 (67%) had a %inh ≥ 30. Four pool samples with 10-30% seropositive animals gave a negative result (Figure 4**Error! Reference source not found.**).

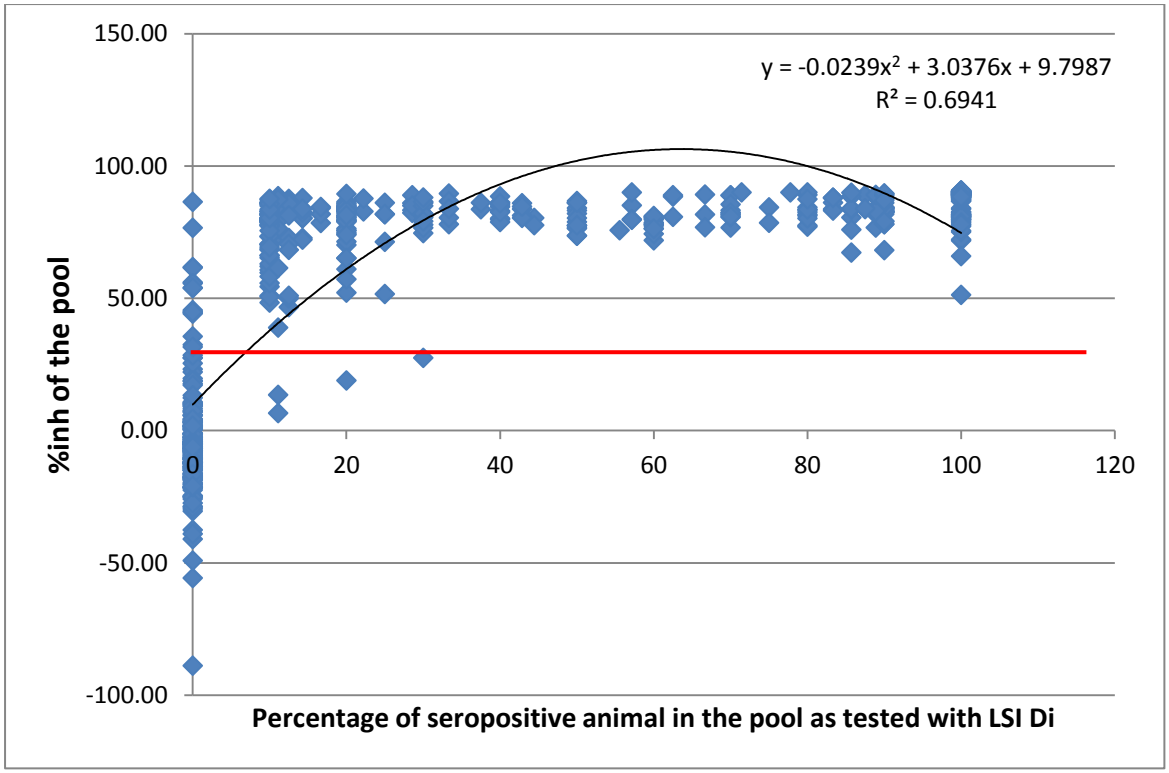

Figure 4: distribution of pool results in %inhibition when tested by LSI Dp in relation to the percentage of seropositive animals in the pool. The red line represents the cut off for the pool protocol of the kit.

6.2 Virological test results

There were 32 herds where all five animals had been tested negative for BVDV antibodies. One of the animals in each herd was randomly selected for antigen testing. A total of 32 samples were tested for BVDV antigen, all with negative results.

6.3 Green house gas (GHG) emissions

Impact of BVDV on the carbon footprint of the Northern Ireland dairy industry

A preliminary analysis has been undertaken to evaluate the potential impact of BVD on the NI dairy industry. The analysis is based on the NI dairy cow population (2011 DARD census) in combination with a number of assumptions. The impact of a range of scenarios has been examined.

The basis of the evaluation is that eradication of BVD will (1) reduce mortality and improve reproductive performance thereby reducing the relative number of replacements required in the Northern Ireland dairy industry and (2) increase the level of milk production per cow.

Assuming no change in milk production output or the level of input per animal but taking into account total land, fertiliser and feed requirements, a 3% reduction in replacement rate resulted in a 1.5% reduction in CO_2e emissions per year which is valued at approximately £1.4 million per annum. Based on projected Carbon trading prices in 2030 (DECC, 2011) this saving would almost double to £2.65m per annum. This only takes into account the value of CO_2e savings although improvements in farm profitability are also likely as a result of lower replacement rates (e.g. reduced AI costs, less heifers to feed/house etc., land freed up for non heifer enterprises etc.).

Assuming no change in total NI milk production and no change in the level of concentrate input per animal, a 2 % improvement in milk production per animal resulted in a 1% reduction in CO_2e emissions. This reduction in CO_2e emissions is valued at £0.97 million per year. A 5% improvement in milk production per cow whilst maintaining the same level of industry output would result in a £2.37million in carbon saving.

Combining a 2% improvement in milk production per animal with a 3% reduction in replacement rate would result in CO_2e savings equivalent to £3.64 million/year from the dairy industry alone (£40/t CO_2e).

Impact of BVDV on the carbon footprint of the Northern Ireland beef industry

GHG emissions from the Northern Ireland beef industry have been estimated at 2.9 million tonnes (Dawson et al 2009). Based on the analyses of the dairy sector, it is estimated that a 3% improvement in replacement rate will lead to a 1.5% reduction in GHG emissions. This amounts to an estimated 43,500 tonnes of carbon equivalents estimated at £1.74 million. Individual animal performance would also likely increase resulting in increased growth rates and a reduced age at slaughter for BVD free beef cattle. These improvements although not currently modelled would likely reduce total CO_2e emissions from NI beef production.

7 Discussion

There is a limited amount of information on the prevalence of BVDV in Northern Ireland. A survey of BVDV antibody titres in 929 bulk tank milks concluded than 89.3% of the herds presented moderate to high levels of antibody and estimated the annual incidence risk for new infection with BVDV to be in the range of 0.133 to 0.477 (D. A. Graham et al. 2001). In England and Wales, a survey of 1070 dairy herds deduced that 65% of the herds were likely to have experienced recent infection with BVDV (Paton et al. 1998). A more recent NI study financed by Agrisearch, tested bulk tank milk samples from 181 dairy herds for antibodies to BVDV and for BVD virus by PCR (unpublished). On the antibody test, results in the majority of herds (95%) suggested that a moderate to high number

of lactating animals within these herds had been exposed to BVD virus. BVD virus was detected in 18 bulk milks (10%) when tested by PCR.

Testing of young stock for BVDV antibodies is recognized as a cost effective method to detect the exposure to BVDV in a herd. Antibodies to BVDV after infection are long-lasting (Fredriksen et al. 1999). Therefore, antibody levels in a bulk milk sample may take a few years after infection to decrease significantly. However, it will only take a few months after the removal of the last PI animal for the young stock to become antibody negative after the maternal derived antibodies have disappeared. This assumes that the young stock animals have been in direct contact with the PI animal. Although the majority of PI animals would die before they reach 24 months of age, a cause for error in the interpretation of the young stock test could be the sampling of animals which are not representative. It is important to exclude purchased animals and to include animals from each separately management group. We tried to record this information in the study questionnaire and also by educating the blood testers and asking them to sample animals from different groups if present and returning this information to the laboratory.

Vaccines for BVD currently commercialised in UK are inactivated vaccines which in some cases have been shown not to induce detectable antibodies by ELISA against the structural protein p80 (NS3) (D. a Graham et al. 2003).

A recent study in Scotland found that 69% of the herds had no recent exposure to BVD virus (Brulisauer et al. 2010). This lower prevalence favours the Scandinavian approach where herds are initially tested by spot test of young stock or bulk milk antibody testing and only those with evidence of exposure undergo follow up testing (whole herd individual screening), to identify and remove PI animals. The prevalence levels that this study has found in Northern Ireland imply that following this approach, 62-72.5% of the herds would need to undertake follow up testing. A more direct approach, such as the Swiss approach (Presi & Heim 2010) where animals are tested for BVD virus, would be more appropriate for the current situation in NI. On top of the high seroprevalence, other similarities with the Swiss situation are the high density of farms as well as the high level of contact between farms. The testing of young calves for virus has also the advantage to give in case of a negative result, a result for the corresponding dam.

A BVD eradication programme for NI would only happen with the full support from the industry. In order to avoid non-compliance and maintain motivation, quick progress is needed. It took ten years for Scandinavian countries to obtain freedom from BVDV. Their programme was aimed at eradication of the virus without the use of vaccination with initially voluntary programmes that had to become compulsory before eradication was achieved (Hult & Lindberg 2005) (Lindberg et al. 2006). In Orkney, a voluntary programme based on the Scandinavian approach was established in

2001. By 2008 it was reported that little progress had been made for the previous 2 years due to lack of legislation and non-compliance (Truyers et al. 2010).

The eradication of BVDV from NI dairy herds, based on combining a 2% improvement in milk production per animal with a 3% reduction in replacement rate would result in CO_2e savings equivalent to £3.64 million/year from the dairy industry alone (£40/t CO_2e). Based on the analyses of the dairy sector, it is estimated that a 3% improvement in replacement rate in the beef industry will lead to a 1.5% reduction in GHG emissions. This amounts to an estimated 43,500 tonnes of carbon equivalents estimated at £1.74 million. The Climate Change Act 2008 is a UK legislation that extends to Northern Ireland and provides a legal framework to reduce emissions of green house gases by at least 80% below 1990 levels by 2050. Agriculture accounts for around 8% of all UK emissions. The savings obtained from the eradication of BVDV in NI would make a big contribution to DARD's Greenhouse Gas Reduction Strategy and Action Plan, and the commitment to meeting targets for reduction in emissions.

8 Conclusions

Seropositivity to BVD virus in Northern Ireland herds is very widespread, showing 67.37% of herds with at least one seropositive animal within the young stock (62-72.5%). This result supports the current design of the NI BVD eradication programme.

Pool serum samples may be a useful tool to determine exposure status of herds. This information will be useful to design the serological phase of the NI BVD eradication programme.

It is estimated that the eradication of BVDV from NI herds would result in CO_2e savings equivalent to £5.38 million/year.

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