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Difficulties arising from the variety of testing schemes used for Bovine Viral Diarrhoea Virus (BVDV)

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Abstract (200 words or less)

Globally, the eradication of bovine viral diarrhoea virus (BVDV) is still in its infancy but eradication has been, or is being, adopted by several countries or regions. Comparisons between countries' schemes allow others to assess best practice, and aggregating published results from eradication schemes provides greater statistical power when analysing data. Aggregating data requires that results derived from different testing schemes be calibrated against one another. We aimed to evaluate whether relationships between published BVDV test results could be created and present the outcome of a systematic literature review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The results are tabulated, providing a summary of papers where there is potential cross-calibration and a summary of the obstacles preventing such data-aggregation. Although differences in measuring BVDV present barriers to academic progress they may also affect progress within individual eradication schemes. We examined the time taken to retest following an initial antibody BVDV test in the Scottish eradication scheme. We demonstrate that retesting occurred quicker if the initial not negative test was from blood rather than milk samples. Such differences in the response of farmers/veterinarians to tests may be of interest to the design of future schemes.

Word Count: 199

Introduction

Programs designed to eradicate bovine viral diarrhoea virus (BVDV) appear, overall, to be making progress, and they exist in various countries (AHI 2014, AHWNI 2014, Barrett and others 2011, Graham and others 2014, Lindberg and others 2006, Presi and others 2011, Sandvik 2004). Other programs are also planned (Farmers Weekly 2014, Laurens, 2014). The variation in the design of these existing programs is of use in designing future programs because it allows comparison of different strategies and lessons to be learnt. The publication of results from different programs around the world also provides a potential opportunity for combining data or results to increase the statistical power when testing scientific hypotheses (Egli 2014).

In Scotland, the eradication of BVDV is on its fourth stage (Scottish Government 2014a). The majority of eradication programs attempt to split herds into ‘possibly infected’ and ‘uninfected’ (Lindberg and others 2006, Prezi and others 2011). In Scotland the two categories are labelled as ‘not negative’ and ‘negative’. Negative herds continue to be monitored in case their status changes. Not negative herds should initiate further testing, commonly trying to find persistently infected (PI) animals and have their movements restricted (Scottish Government 2014b).

Within the Scottish BVDV eradication scheme there are eight possible testing methods (including three types of calf sampling) and eight groups of laboratories that can process the tests (Scottish Government 2015a, Scottish Government 2015b). Samples may be tissue, semen, blood or milk and can be tested for antigen or antibody (Scottish Government 2014c).

For dairy herds, bulk milk samples are common in eradication schemes to identify antibody status with antigen blood testing frequently used to establish whether the herd is currently infected and to find PI animals. For beef herds, blood testing predominates. The difference between common types of bulk milk and blood tests provides a useful example of the ramifications of differences in tests.

Within each sampling method (as described above) there are also many different laboratory tests for BVDV (Lanyon and others 2014a) and a comprehensive list of those available is beyond the scope of this paper. Many of the tests available are based on detection of the virus or the antibody to the virus, and use enzyme-linked immunosorbent assay (ELISA), immunohistochemical tests, reverse transcription polymerase chain reaction (RT-PCR), or neutralization serum antibody tests (Radostits and others 2007). The variation in test regime depends upon a combination of the particular kit manufacturer, the sampling regime and the tissues sampled.

Between eradication schemes, variety provides a series of “natural experiments” in which different schemes adopt different tests and, thus, the international community has evidence regarding their relative merits. However, this depends on how the tests and their results are reported, and people in business rarely have time to dwell on the complexities of test performance (e.g. sensitivity and specificity). The use of different tests is not only important to the researcher, but also to the farmer, veterinarian and the eradication scheme.

We set out to try to establish relationships between reported BVDV test results. The first step in this was a systematic literature review using the Preferred Reporting Items for Systematics Reviews and Meta-Analyses (PRISMA) guidelines (Moher and others 2009). We present the results of this review followed by a discussion of the difficulties we had in using the results to establish a relationship between tests. As a consequence of the difficulties we encountered, we also examined the test results from the Scottish BVDV eradication scheme to see if the industry treated results from blood and milk tests differently. We present the results of these tests and a discussion surrounding the differences between blood and milk tests.

Materials and Methods

Systematic Review

In order to find as many as possible, in a repeatable and documented way, of the papers that have published results that would help us compare test results we conducted a systematic literature review using PRISMA guidelines (Moher and others 2009). Full details of our review process are shown below:

1. An advanced search was made on Web of Science http://webofknowledge.com/
2. The search we used was:

   (TI= ((bovine viral diarr) OR (BVDV) OR(bovine virus diarr*)) AND (TI=(milk OR antibod* OR *prevalence OR eradication OR herd OR elisa)))*
3. The results were initially filtered on web of science by selecting the document type, research domain and language.
   1. Document Types: *Article or Review*
   2. Research Domain: *Science Technology*
   3. Language: *English*
4. The results were exported in tab delimited format to Windows including their abstracts (where possible). The remaining filtering took place in Microsoft Excel.
5. The results which didn't have an abstract exported (AB column) were removed.
6. The results that stemmed from a conference (CT column) were removed.
7. We then removed any results which we could not access electronically or were not available in paper format from previous work. Double entries were removed.

8. Papers were then submitted to two screening questions:
    i. Are numerical results, a statistical model or graphs produced from which the reported results can be read?
    ii. Are multiple tests or multiple testing procedures used? Failing that is there an equation that can be used to compare with other results?

   We retained only those publications where quantitative results (e.g. number of animals positive in herd, number of PI, test scores or percentages) were available for comparison from tables or graphs. The exception was where an equation or model governing the relationship between test results, test types or PI animals was reported.

For each paper we extracted the following information into tabular form: the sample types (blood/bulk milk), the tests used, how the results are presented, whether individual PI animal status was reported, whether the animals/herds tests were vaccinated, and an explanation of why we think the result can or cannot be used to link to another paper/test. As the papers were subjectively assessed there is a risk of bias across the studies from step 8 above and in extracting the results from the publication. Where the lead author was in any doubt, one of the other authors acted as a secondary reviewer.

<u>Retesting Analysis of the Scottish BVDV Eradication Scheme</u>

To establish whether the results from blood and milk antibody tests are treated differently we used the results of the BVD eradication scheme in Scotland. Results were collated and matched by the County, Parish, Holding (CPH) unique identifier. For each CPH we identified the first blood or milk antibody test and then established the number of days for the same CPH to conduct an antigen test. Those antigen tests taking place less than nine days after the antibody test were counted as part of the same testing due to the length of time needed to return test results to the CPH in question. These resampling results are presented as a proportion of those CPHs within each class of the same initial test (milk or blood) and initial test result (negative or not negative). Only the test results recorded as “Negative” or “Not Negative” were used. To use other values would have necessitated a subjective interpretation of the overall test result.

To establish if there was a statistical significant difference in the proportions of holdings retesting within 90 days (AFBI 2015, DEFRA 2015) based on the type of initial antibody test, we carried out a two-sided proportion test and a survival analysis. We selected a 90 day threshold for our analyses because this is the period within which retesting is normally recommended or required for bulk milk tests, regardless of herd status (AFBI 2015, DEFRA 2015). The CPHs were split by initial test type (milk or blood). Those CPHs that did not retest within 90 days, regardless of whether they later retested, were treated as “not retesting”.

For the proportion test (Newcombe 1998) a two by two table of counts (resample before 90 days yes/no versus initial antibody test blood/milk) was constructed before we used the *prop.test* function in the statistical software R (R Core Team 2015) to carry out the test. The survival analysis was carried out using the survival package (Therneau 2015, Therneau and Grambsch 2000).

Results

Systematic Review

Table 1 shows the papers that completed the PRISMA systematic review process. For each paper we show: the sample types (blood/bulk milk), tests used, result format, whether individual PI animal status was reported, the vaccination status of the animals/herds (if reported) and description of whether the result can be used to link to another paper/test. The number of papers that remained after each stage of our process described above are shown in table 2.

The papers in table 1 should allow us to compare results from different tests and different testing methods. However, we encountered significant difficulties in doing so. The most common difficulties surrounded vaccination and test variety.

Retesting Analysis of the Scottish BVDV Eradication Scheme

Figure 1 shows the proportion of farms (in the Scottish BVDV eradication scheme 2013-2014) retesting over the year, split by the type of initial test. (Red, solid: initial negative blood test. Green, short dashed: initial not-negative blood test. Blue, dashed: initial negative milk test. Purple, long-dashed: initial not-negative milk test.) There is a clear difference between the time taken to retest. Within the first 90 days, farms are more likely ($p = 0.05139$ from the two-sided test of proportions) to retest following a not-negative result if the initial test was using blood testing rather than bulk milk. This is confirmed by the survival analysis which provides a relative risk of 0.73 with a 95% confidence interval of (0.54, 0.98) and a rejection of the null hypothesis that the relative risk is one, based on a p-value of 0.037.

Discussion

Whilst progress appears to be being made in the uptake of eradication schemes for BVDV around the world, it is still in its infancy, with more countries ***not*** yet planning such a scheme than there are countries planning, in the process of, or having achieved, eradication (Moennig and Becher 2015). We are therefore at a useful stage in the global eradication trend, because we can make use of data being reported from the different schemes from around the world. Results from schemes can be used either by aggregating data in order to achieve increased statistical power when asking epidemiological questions (e.g. does herd size affect the probability of a herd containing a PI and by how much?) or, qualitatively, by heeding the lessons learnt from the reports of successful and unsuccessful strategies. Here we describe the difficulties we had in aggregating data and present evidence from one particular scheme in which the differences in farmers'/veterinarians' perception of the test may be influencing the time taken to retest. Comparative studies of strategies ***within*** a scheme may be even more powerful than between schemes because unknown confounders (at the scheme level) should be effectively controlled for.

Aggregating data requires that data be "calibrated" into common and genuinely equivalent units (e.g. within herd seroprevalence) and therefore is dependent on comparing results from different schemes with different methods. The difficulties encountered in comparing results from different papers were mainly due to: vaccination, the variety of tests used and how their results were reported. It is clear from table 1 that some papers have vaccinated animals whilst other are unvaccinated or vaccination status is not reported. Some eradication schemes have banned vaccination (Lindberg and others 2006) and comparing antibody results across studies without accounting for differences in vaccination regimes risks ignoring vaccination as a clear confounder (Bauermann and others 2013, Gonzalez and others 2014a, 2014b, Humphry and others 2012, Stevens and others 2011).

Whilst vaccine usage might possibly be dictated by an eradication scheme, (Lindberg and others 2006) the particular laboratory tests used could be more difficult to control. For example, within the Scottish BVDV eradication scheme the testing methods and the laboratories analysing them are controlled but not the manufacturer of the tests they use (Scottish Government 2015a). Table 1 gives an example of the variety of tests for both blood and milk that are reported in the literature. Tests range from those used by specific laboratories to bespoke (Houe 1994, Houe and Meyling 1991, Rüfenacht and others 2000). Some results are reported with insufficient detail to allow comparison. For example, the percentage inhibition results from Booth and others (2013) are reported without the control values needed to replicate them. However, in Niskanen and others (1991) and Niskanen (1993), the control values are reported. Incomplete reporting of results may not be the authors' choice - it may arise from the laboratory or test used. However, where full details can be made available, doing so would assist other researchers and, possibly, those in charge of eradication schemes.

The accuracy and reliability of the type of test should also be considered as this can be used to estimate confidence ranges around any calibrated result from one scheme in comparison to another. For example there is good evidence (Brülisauer and others 2010, Humphry and others 2012) that using the proportion of seropositive young-stock gives better classification of herds into distinct antibody-level groups than bulk milk antibody scores. Figure 2 shows the frequency distribution for percentage positivity (PP) scores of bulk milk tests for 220 Scottish farms whilst figure 3 shows the frequency of 10 young stock that were BVDV seropositive in 274 Scottish herds. Even with the complicating observation of a small spike at about 5 seropositive animals in figure 2 (see Brülisauer and others (2010) for a full discussion) the bloods have a very clear second maximum for 9 and 10 seropositive animals, whereas the bulk milk results have no clear separation.

This suggests that at herd-level, bulk milk results are more likely to produce false positives, or false negatives than is the serum screening of young-stock. Bulk milk antibody scores are not only an average of contributing animals but they also represent an average over time, reflecting historic as well as current BVDV status. The removal of PI animals will not necessarily produce an immediate change in bulk milk results (figure 4 - reproduced from Houe 1999). It is clear that even three years after the removal of the final PI animal, the bulk milk results had not changed greatly.

Differences in the “performance” of a test are not just of importance to academic researchers when trying to make use of reported results from around the world. These differences, whilst appearing highly epidemiological and quantitatively technical, are also of great importance to the individual scheme itself and to the farmers and practitioners within the scheme. How farmers and their veterinarians respond to any difference in test performance (sensitivity and specificity) is hard to predict. A precautionary approach may be adopted – i.e. any bulk milk score which is just negative might be followed up with additional tests lest it be a false negative.

Conversely, a riskier approach might be that any not-negative test be considered a probable false positive. The Scottish Eradication data provides evidence that may be, in part, an example of scheme members responding to different tests according to the different test performance.

Figure 1 shows that the length of time taken to retest from an initial not negative antibody test result is dependent on whether that initial test was a blood or a milk test. It is possible that farmers take a not-negative result from blood more seriously and hence retest quicker. Farmers may be taking the riskier approach with the not negative milk result which we suggested above. We should also consider whether dairy farms treat PI calves with less concern than beef farms as calves are removed from dairy herds at a younger age than in beef herds. Other explanations include the availability of follow up tests depending on whether the farm is beef or dairy, if the financial impact of a movement ban of animals is greater for beef farms or if there is more pressure within the beef sector for retesting.

We do not know why those receiving not negative blood test results retest quicker but the discussion about bulk milk results is pertinent not least because the Scottish Government have removed the option for bulk milk tests from June 1st, 2015 (Scottish Government 2015c). This seems understandable as eradication enters its next phase, given the importance of successful detection of the virus and the relative imperfection of the bulk milk antibody test but this policy differs from some other schemes (Hult and Lindberg 2005).

Whilst we have acknowledged the limitations of bulk milk testing, this is not to dismiss the value of testing milk from all or some cows in a herd. Milk sampling from a sub-group of milking animals can be particularly useful for testing new heifers whose antibodies provide a “signal” of recent rather than historic infection (Brownlie and Booth 2014, Houe and others 2006, Ohlson and others 2013). It is therefore reasonable that, after a scheme has effectively eradicated BVDV (and depending on vaccination regimes), the relatively convenient and cheap test that is bulk milk testing may come into its own as a first line of screening for sporadic breakdown (Booth and others 2013).

There are many different tests available for BVDV and a lot of research has been published detailing test results and progress in eradication schemes (Laurens 2014, Lindberg and others 2006, Sandvik 2004). Whilst not of immediate concern to the design of a scheme, taking into account how transferable the results of that scheme are with data from other schemes has the added benefit of facilitating research based on aggregating results. When designing an eradication scheme the testing methods and individual tests available should be considered to ensure that a variety of tests within the scheme does not discombobulate the scheme itself. If the scheme is too complicated, this will only hinder the eradication.

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Table 1: Details of the papers from the PRISMA systematic review. The table shows the bulk milk and blood tests used, the results format, whether or not the PI status of individual animals was reported, whether the herds/animals tested were vaccinated or not and if the authors think it possible to link the results to another test or paper. (Abbreviations other than company names, not used previously: Ab – antibody, Ag – antigen, IPMA – immunoperoxidase monolayer assay, OD – optical density, PP – percentage positive, VNT – virus neutralisation test, VI – virus isolation)

Paper	**Bulk Milk (*Test*)**	**Bloods (*Test*)**	**Results reported as:**	**PI status reported**	**Vaccinated / Unvaccinated**	**Is link to another paper possible?**
Ahmad and others 2014	No	Yes – (Herdcheck IDEXX *Antigen Capture Elisa tested against immunochemisitry and RT-PCR)*	Comparison of positive results across tests	No	No mention of vaccination.	Difficult to link as no sample values given for the sample to positive ratio
Beaudeau and others 2001a	Yes – (*LSI BVD/BD p80 blocking ELISA*)	No	Equation relating herd prevalence to percentage inhibition	No	82 of 112 used in equation no history vaccinated	No control values reported and percentage inhibition defined by OD control
Beaudeau and others 2001b	Yes – (Pourquier BVD/MD p80 milk ELISA)	Yes – (VNT on matched sera)	Comparison of OD (%) from ELISA and the VNT titre. Plot of OD (%) vs within-herd prevalence of positive animals.	No	Nothing reported.	As with previous paper, cannot read the percentages off the graphs and no equation given for relationship between OD and within-herd prevalence (unlike first Beadeau paper).
Beaudeau and others 2001c	Yes – (LSI BVD/DB p80 blocking ELISA)	Yes – (VNT and LSI BVD/BD p80 blocking ELISA)	Comparison of percentage inhibition from ELISA of serum vs VNT titre of same sample. Comparison of percentage inhibition from ELISA of bulk milk and VNT of matched serum.	No	No specific reporting of number of vaccinated herds. Makes point that "cut-off values provided by the ROC analysis were insensitive" to vaccination.	So many results that can't read the percentage inhibition from graphs for the fixed titres. Provides cut off of 50% inhibition as +/-.
Booth and others 2013 (using Booth and Brownlie 2011 for additional information)	Yes – (*AHVLA indirect ELISA – claimed to be "broadly comparable" to SVANOVA indirect ELISA*)	Yes – (*Antigen ELISA if >6months, pooled PCR if <6months*)	OD ratio, PIs, percentage BM contributors' positive, negative.	Yes	Two farms that didn't vaccinate.	No control values given, no precise calculation. Sensitivity and Specificity given in Booth and others 2011 approximate.
Bosco Cowley and others 2012	Yes – (*Svanova ELISA-Ab*)	Yes – (*Svanova ELISA-Ab on pooled samples*)	Only contingency table for relationship between blood and milk results. PP from pooled serum vs % of positive samples plotted. Polynomial relationship.	No	All analysis applied to unvaccinated herds	Polynomial relationship between PP and % positive samples not published. Need full results rather than contingency table.
Cornish and others 2005	No	Yes – (Syracuse Bioanalytical Inc. Ag ELISA with VI and RT-PCR to confirm)	Table of PI animals with results from VI, RT-PCR and ELISA with VN titre.	Yes	All dams of calves tested were vaccinated.	Could compare with similar tests on calves.
Diéguez and others 2008	Yes – (Pourquier BVD/MD p80 blocking ELISA)	Yes – (Pourquier BVD/MD p80 blocking ELISA & IDEXX ELISA antigen serum plus BVD test kit)	Plot of herd seroprevalence vs % inhibition in BTM.	Tested for but results not tabulated or plotted.	Yes	No explicit results given, just cut-offs. Could compare with other papers using the same tests if we could get the complete results.
Eiras and others 2012	Yes – (*Pourquier BVD/MD/BD p80, Civtest bovis BVC p80, IDEXX HerdChek*	Yes – (*Pourquier BVD/MD/BD p80 & IDEXX antigen serum kit*)	Threshold of transformed optical density values of all four bulk milk tests compared against thresholds	No	24% of herds have been vaccinated 78/325.	No control values given and some herds vaccinated. If all sample results provided then a relationship between the tests might be established.

	BVDV, SVANOVA Svanovir BVDV-Ab)		established from blood tests.			
Foddai and others 2015	Yes – (Danish blocking ELISA (Bitsch and others 1997) & SVANOVA BVDV-Ab ELISA)	Yes – (Danish blocking ELISA (Bitsch and others 1997) & SVANOVA BVDV-Ab ELISA)	Graphs of PP vs blocking % for both milks and bloods.	No	No mention of vaccination.	Could compare to others using the same test. Numbers little difficult to read accurately from plots.
Graham and others 2003	Yes – (SVANOVA indirect BVDV-Ab ELISA & LSI blocking ELISA NS2-3)	Yes – (SVANOVA indirect BVDV-Ab ELISA & LSI blocking ELISA NS2-3 & VNT described in Graham and others 1997)	Mean results of tests from groups before and after vaccination. Plots (with best fit lines) of Indirect ELISA COD against VN titre and % inhibition of blocking ELISA	No	All animals vaccinated during testing	If the lines of best had been published they could be used to compare the results of Indirect ELISA COD with either VN titre or blocking ELISA – if other animals have used the same vaccines.
Hanon and others 2014	No	Yes – (ADIAGENE, Adiavet BVDv RRT-PCR test & IDEXX BVDV Ag/serum plus ELISA)	Predicted probability of PI animal based on the C_t value from the RRT-PCR.	Yes	No mention of vaccination.	If the equation or similar for the model had been reported then could find the probability of PI from any other study using the same test.
Houe 1994	Yes – (*Indirect ELISA*)	Yes – (*Meyling's own test*)	Virus positive ~ ab positive ~ antibody titer in BM (related to OD)	Yes	Unvaccinated	There are no explicit details provided on the indirect ELISA used but could link with Houe and Meyling 1991 as it too uses Meyling's test.
Houe and others 1995	No	Yes – (IPMA (Meyling 1984) & VNT	Tabulated values % of antibody positive vs mean/median antibody titer results.	Yes	Mixture of vaccination history.	Could relate to other herds using those tests with the same vaccination history.
Humphry and others 2012	Yes – (*SVANOVA indirect ELISA (percentage positive), the test from Drew and others 1999 and SVANOVA indirect ELISA (corrected OD)*)	No	percentage positive OD Corrected OD All linked to Swedish classes	No	Unvaccinated	Could possibly link this to other papers using the same tests.
Kuta and others 2013	Yes – (SVANOVA BVDV-Ab ELISA and SVANOVA BVDV-Ab ELISA confirmation format)	No	Correlation of COD values from initial ELISA and PP values from confirmatory ELISA for 28 herds that were double tested.	Yes	Unvaccinated	Could be used to compare COD with PP from SVANOVA ELISA tests – providing the confirmation test is essentially the same as original.
Lanyon and others 2013	No	Yes – (IDEXX BVDV Total Ab and VNT Titre	Regression equation linking VNT titre result and sample to positive ratio of ELISA	No	Unvaccinated	Could be used to compare other VNT Titre results and ELISA results using the same test.
Lanyon and others 2014b	Yes – *(IDEXX BVDV Total Ab Test)*	Yes – *(IDEXX BVDV Total Ab Test and RT-PCR)*	Percentage of herds testing positive / negative for both blood and milk tests. Relationship between milk and blood sample to positive ratios also presented.	No	No mention of vaccination	Relationship of bulk milk to serum results could provide link if the same test has been used.
Muvavarirwa and others 1995	No	Yes – (ELISA (as described by Howard and others 1985) & serum neutralisation test)	Comparison of results from ELISA and values of SN titre	No	No mention of vaccination	Could compare with results from the same test or with SN titre.

Niskanen 1993	Yes – (*SVANOVA indirect ELISA*)	Yes – (*SVANOVA indirect ELISA*)	Absorbance value ~ percentage prev of ab positive lactating cows in herds.	No	Unvaccinated	Should link to any other SVANOVA indirect ELISA (control values given)
Niskanen and others 1991	Yes – (*SVANOVA indirect ELISA*)	Yes – (*SVANOVA indirect ELISA*)	# ab positive ~ absorbance values of bulk milk.	PIs were removed	Unvaccinated	Should link to any other SVANOVA indirect ELISA (control values given). PIs removed in between two of the yearly results.
Rüfenacht and others 2000	Yes – (*Authors' own ELISA*)	Yes – (*Antigen capture ELISA (Strasser) and RT-PCR (Wirz)*)	Herd abpositive prevalence ~ Antigen ELISA positive (1st and 2nd tests) /ab ELISA positive /RT-PCR positive	Yes	Unvaccinated	Test is author created so very difficult to link to a more widely used test.
Sandvik and Krogsrud 1995	No	Yes – (SVANOVA ELISA ab test, Moredun antigen test)	Tabulated values of antibody OD vs antigen OD.	No	Unvaccinated	Could possibly be used to create link between SVANOVA test and antigen results.
Schreiber and others 1999	No	Yes – (Specific test unclear)	Table of number of animals in herd with number of seropositive and PI per herd	Yes	37% vaccinated but type of vaccine produces few to none antibodies	Can link between other herds with PI animals although specific test used unclear.
Ståhl and others 2008	Yes- (SVANOVA indirect ab ELISA)	Yes – (IDEXX BVDVB Ag/Serum)	Table of number of positive spot blood tests and mean OD for the bulk milk tank.	Yes	7 out of 55 herds vaccinated	Could create link between bulk milk and number of positive young stock if same tests used.
Taylor and others 1995	No	Yes – (Bespoke Ab ELISA with VNT and PCR)	Table of each pen with titre, ELISA, VN and PCR results with number of animals tested per pen.	Yes	No specific mention of vaccination for BVDV.	Difficult to compare as the ELISA created was bespoke.
Vanderheijden and others 1993	No	Yes – (Would appear to be bespoke p80 ELISA test & SN)	Table comparing SN titre vs p80 results.	Yes	2 tests specifically designated as vaccinated or unvaccinated.	Could compare with other results from same test.
Weir and others 2013	Yes – (IDEXX ELISA)	Yes – (IDEXX ELISA)	Plot of milk S/P ratio vs serum S/P ratio with regression line.	No	Unvaccinated	Could be used to compare results from previous tests if regression line had been provided.
Zimmer and others 2002	Yes – (Ceditest BVD blocking ELISA)	Yes – (Ceditest BVD blocking ELISA)	Proportion of herd ab+ vs status of herd from btm	Yes	No mention of vaccination.	Could be used to compare with btm results using the same test.
Zimmer and others 2004	No	Yes – (Antibody test via VNT, Antigen test via VI, Synbiotics ELISA and RT-PCR	Table showing results for each calf tested – titre vs antigen test and RT-PCR and serological titre.	Yes	No mention of vaccination	Useful comparison with other studies on calves and using the same tests.

Table 2: The number of papers still available to the authors after each step of the PRISMA systematics review

Step of PRISMA	1- Web of Science	2 - Search Terms	3 - Filter Results	5 - Remove No Abstract	6 - Remove Conference Papers	7 - Collect Papers	8 - Screening Questions
Number of Papers remaining following that step	$> 10^9$	654	508	386	352	259	28 electronic and 2 non-electronic.

Figure 1: Time taken (days) from an initial antibody test to retesting for antigen, separated by initial antibody test type (milk and blood) and result (negative/not-negative). (Red, solid: initial negative blood test. Green, dotted: initial not-negative blood test. Blue, dashed: initial negative milk test. Purple, long-dashed: initial not-negative milk test.) Data is taken from the Scottish BVDV eradication scheme 2013 – 2014.

Figure 2: Frequency distribution of percentage positivity results from 220 BVDV bulk milk tests from a survey of 220 Scottish Farms. Previously published in Humphry and others 2012.

Figure 3: Number of seropositive animals from 10 young stock sampled in a survey of 274 Scottish herds. Previously published in Brülisauer and others 2010.

Figure 4: Plot of the blocking percentage of antibody reaction in a bulk milk tank against days after the removal of the last persistently infected animal. A Linear regression line is also shown. Reproduced with permission from Houe 1999.