



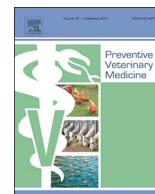
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Quantifying the role of Trojan dams in the between-herd spread of bovine viral diarrhoea virus (BVDv) in Ireland

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ABSTRACT

A compulsory national programme to eradicate bovine viral diarrhoea virus (BVDv) began in Ireland on 1 January, 2013. The objective of the current study was to quantify the role of Trojan dams (animal(s) not persistently infected (PI) with BVDv but carrying PI foetus(es) and introduced to the herd while pregnant with the PI foetus(es)) in the farm-to-farm spread of BVDv in Ireland, and to identify herd-level risk factors for producing or introducing a Trojan dam. The study population included all BVD+ calves born in Ireland between 1 January, 2013 and 31 December, 2015, along with their dams. BVD+ calves included all calves on the national programme database with an initial positive or inconclusive virus test, without a confirmatory re-test (status BVDPOS) and those with an initial positive or inconclusive test and a positive confirmatory test (status BVDPI). The Trojan status of dams was determined after considering their history of movement and of potential BVDV exposure, relative to a defined window of susceptibility (WOS; days 30–120 of gestation). During 2013–15, there were 29,422 BVD+ birth events to dams that were not themselves BVD+, including 2526 (8.6%) most-likely attributable to Trojan dams. The percentage of these birth events attributable to Trojan dams was significantly different ($P < 0.001$) between years, being 7.1% in 2013, 9.2% in 2014 and 10.6% in 2015. During 2013, in 9.9% of herds with one or more BVD+ birth to non-BVD+ dams, at least one of these births was attributed to a Trojan dam. In 2014 and 2015, the percentages were 11.8% and 13.3%, respectively. In 2013, in 7.8% of herds with one or more BVD+ birth to non-BVD+ dams, all of these births were attributable to Trojan dams. In 2014 and 2015, the percentages were 9.2% and 10.7%, respectively. A logistic GEE regression identified dam parity, herd size and an interaction between herd type and season as significant predictors for the birth of a BVD+ calf to a Trojan dam. Significant predictors for the sale of a Trojan dam from BVD+ herds included those selling more than one pregnant female and those with more than 2 BVD+ animals in the herd. Introduction of pregnant adult females is a potential source of BVD+ births in BVD-free herds and may add to the burden of infection in non-BVD-free herds. Addressing this route of transmission will be critical for herds that are now free of infection and wish to continue to purchase animals without introducing it.

1. Introduction

Bovine Viral Diarrhoea (BVD) is an economically important disease of cattle (Stott et al., 2012). National programmes to eradicate BVD virus (BVDv), the cause of BVD, are underway or have been successfully completed in several Member States of the European Union (EU). Using programmes based on serological screening of herds, several countries, including Norway, Sweden and Denmark, have proved that eradication can be achieved. For example, the national herd in Norway has been considered BVDv-free since 2007 (Norström et al., 2014). Using a

different approach, based on tissue tag testing, to directly detect persistently infected (PI) calves, the prevalence of PI births in Switzerland was reduced from 1.8% in 2008 to less than 0.2% in 2010 (Presi et al., 2011), and a herd-level prevalence of 0.12% was reported in 2014 (Schärerer et al., 2015). In Ireland, a compulsory, industry-led, national BVDV eradication programme began in 2013, preceded by a voluntary programme in 2012 (Graham et al., 2014b). This requires a tissue tag sample from all newborn calves to be tested for BVDv. Negative test results for calves are used to assign an indirect negative (INDINEG) status to the dam on the programme database, provided by the Irish

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Cattle Breeding Federation (ICBF), while a positive result creates a suspect (DAMPI) status for the dam. National legislation (S.I. No. 30 of 2017; Anon, 2017) prohibits the movement of animals born during the programme, and of all suspect animals, independent of their year of birth, other than to slaughter, in the absence of a negative result. Between 2013 and 2014, the percentage of approximately 83,000 breeding herds in Ireland recording one or more positive or inconclusive result from tissue tag testing dropped from 11.3% (9497) to 7.6% (6178), reducing further to 5.9% (4752) in 2015 and 3.2% (2530) in 2016. Over this same period, the annual prevalence of PI births decreased from 0.66% to 0.16% (Animal Health Ireland, 2017). Of the calves born in Ireland during the life of the eradication programme, 0.66% were PI in 2013, 0.46% in 2014 and 0.33% in 2015 (AHI programme results).

PI animals play a central role in the epidemiology of BVD. Direct contact with PI animals is the most efficient means of transmitting the virus to susceptible animals (Niskanen et al., 2002), whereas transiently infected (TI) animals are considered to be of minor importance (Niskanen et al., 2002; Nickell et al., 2011; Sarrazin et al., 2014). Cattle become PI as a result of infection *in utero* during a window of susceptibility (WOS) between approximately 30 and 120 days of gestation (Lanyon et al., 2014), and shed high levels of virus in all bodily secretions throughout their lifetime (Brownlie et al., 1987).

The prevention of between-herd spread is a central aspect of national BVDV control and eradication programmes. Trade in non-PI dams pregnant with PI foetuses (so-called Trojan dams) is a potentially important route of between-herd spread, particularly if the trade in PIs is otherwise controlled (Lindberg et al., 2001). Therefore, measures to limit the movement of Trojan dams can contribute to national eradication programmes. In Sweden, for example, herds under restriction due to evidence of ongoing or recent infection based on herd-level serological testing were not allowed to sell pregnant animals other than to slaughter, unless the herds had been tested twice for antibodies with an approved result (Hult and Lindberg, 2005). In the Swiss eradication programme, all pregnant females on a farm where BVDv was detected were restricted to that holding until after their next calving (Presi et al., 2011).

Some limited data are available quantifying the relative importance of different transmission pathways in between-herd spread, including the movement of Trojan dams. Alban et al. (2001) used Danish data from 1995 to 99 to determine how and when a herd was initially infected. While limited to some extent by the availability of data, particularly for herds outside the Milk Recording Scheme (MRS), they reported that new infections on 2.4% and 4.3% of non-MRS and MRS herds, respectively, were due to movements of dams in late gestation (> 120 days) into these herds. They concluded that transfer by trade in pregnant animals was not an important transmission route for BVDV in Denmark during this period.

Trojan dams are also recognised as a disseminator of infection in Ireland. However, as yet there has been no published work either describing the characteristics of these animals, the herds from which they are moved and into which they are introduced, or quantifying their impact on the national eradication programme. The availability of national databases with details of births, movements and BVD statuses for all animals provides a robust means of assessing their contribution to the spread of infection. Therefore, the objectives of the current study were to quantify the role of Trojan dams in the farm-to-farm spread of BVDv in Ireland, and to identify herd-level risk factors for producing or introducing a Trojan dam.

2. Materials and methods

2.1. Definitions

A number of definitions are used in this paper, as outlined below:

BVD+ animal. BVD+ animals included all animals, of any age

identified as either BVDPOS (animals with an initial positive or inconclusive virus test but without a confirmatory re-test being conducted) or BVDPI (animals with an initial positive or inconclusive test and a positive confirmatory test) on the national programme database. BVD+ animals were considered PI for the purposes both of the study and more widely within the national programme.

Trojan dam. The dam of a BVD+ calf was considered a Trojan dam if she satisfied each of the following conditions: the dam had been introduced to the birth herd (as defined below) while pregnant, the dam was carrying a BVD+ foetus on introduction and the dam was not herself BVD+ (see Section 2.3 for further details of determination of dam status).

A non-Trojan dam. This status was applied to any dam giving birth to a BVD+ calf where evidence indicated that infection leading to the development of the BVD+ calf occurred in the herd in which that calf had been born, or any dam that was BVD+ giving birth to a BVD+ calf.

Trojan birth. The birth of a BVD+ calf (or calves, if there were multiple births) from a Trojan dam.

Study animals. All BVD+ calves born in Ireland between 1 January, 2013 and 31 December, 2015 (so-called study calves) and their dams (study dams).

Study period. 1 January, 2013 and 31 December, 2015 inclusive.

The window of susceptibility (WOS). The stage of gestation during which transient infection of a dam with BVDv was likely to lead to the development of a PI calf. In this study, the WOS was assumed to be between 30 and 120 days of gestation (Brownlie et al., 1998).

The birth herd. The herd into which a BVD+ calf was born.

The source herd. population included any herd which had a PI animal present in 2014.

The herd of origin. The birth herd of the dam. The herd of origin was used to determine whether heifers or cows might have left their birth herd and returned in-calf (e.g. following contract rearing) as a Trojan dam.

Associated herds. The birth and source herds of each Trojan dam of a BVD+ calf.

2.2. The data

Relevant data about the study calves, the study dams, and associated herds were obtained from the national programme database, based on the results of tissue tagging calves and ancillary blood tests, and from the national Animal Identification and Movement (AIM) database, as follows:

About the BVD+ calves:

- **The calf:** animal ID, date of birth, date of death, BVD status (BVDPOS or BVDPI).
- **The dam:** animal ID, date of birth, parity, current BVD status, whether she entered the birth herd within 282 days of calving and, if so, the date of entry, import status.
- **The birth herd:** herd ID, regional location (as defined by the Irish Regions Office; www.iro.ie), total herd size on 1 January in 2013, 2014 and 2015, enterprise type (dairy, beef or dual purpose).

About selected dams (all study dams that had entered the birth herd within 282 days of calving and were not themselves known to be BVD+):

- **The dam:** the number of times she moved during the pregnancy resulting in the Trojan birth, including whether or not she moved through a cattle market (if so, also the market ID and associated dates).
- **All source herd(s):** herd ID, herd size, herd location by county, herd enterprise type.

About all source and birth herds associated with those study dams

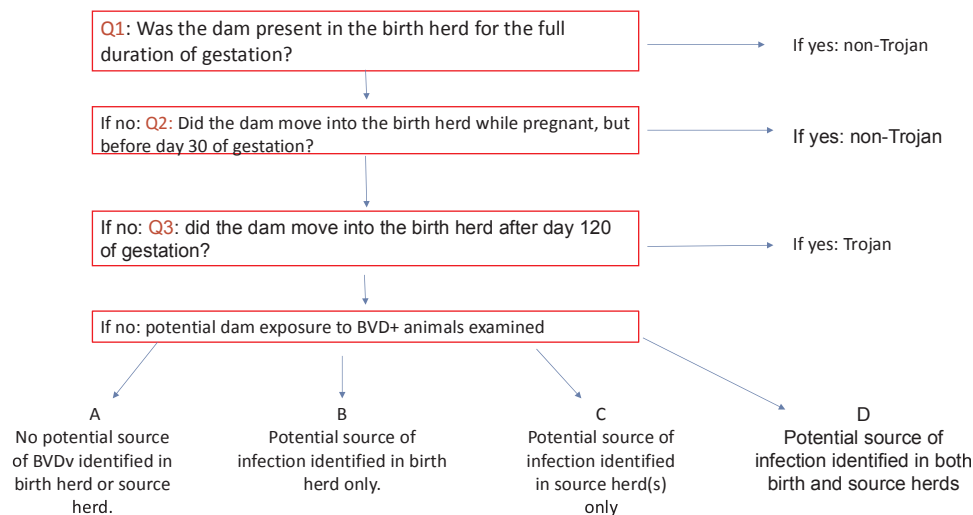


Fig. 1. Decision tree used to identify dams of BVD+ calves as Trojan or non-Trojan.

that entered the birth herd less than 282 days before the birth of a BVD+ calf:

- **BVD status** of all non-negative animals (i.e. those that had either a positive or an unknown status on the national database) that were present in the herd at any time during the WOS of the Trojan dam.
- **Dates** of entry to and exit from the herd of each of these animals.

2.3. Determining Trojan dam status (see Fig. 1)

Firstly, BVD+ dams were excluded. Then, the following decision-tree was used to determine the Trojan status of each study dam:

1. Was the dam present in the birth herd for the full duration of gestation?
 - a) If yes, the dam was not introduced during this pregnancy and could only have encountered BVDv while in this herd. Defined as *non-Trojan*.
 - b) If no, go to Q2.
2. Did the dam move into the birth herd whilst pregnant, but before day 30 of gestation?
 - a) If yes, then the dam entered the birth herd before the WOS had started. Defined as *non-Trojan* (based on published literature outlined below).
 - b) If no, go to Q3.
3. Did the dam move into the birth herd after day 120 of gestation?
 - a) If yes, then the dam entered the birth herd after the WOS had ended. Defined as *Trojan*.
 - b) If no (entered during the WOS, allowing the possibility of infection in either a source herd or the birth herd), go to Q4.
4. The dam moved into the birth herd at some point between days 30 and 120 of gestation. Where was the dam exposed to BVDv, based upon data available on BVD+ animals?
 - a) No potential source of BVDv identified in either the birth or source herd(s) during the WOS.
 - b) Potential source of infection identified in birth herd only.
 - c) Potential source of infection identified in source herd(s) only.
 - d) Potential source of infection identified in both birth and source herds.

The Trojan status of dams that moved into the birth herd at some point between days 30 and 120 of gestation (those identified in Q4) could not be determined conclusively, based on the data available. On balance, 4b dams were most-likely non-Trojan and 4c dams most-likely Trojan, whereas the Trojan status of 4a and 4d dams was uncertain.

Here, we report three different estimates of the number of Trojan dams, including:

- the minimum number of Trojan dams (all dams identified in 3, based on the assumption that all dams entering the birth herd between days 30 and 120 of gestation were infected in the birth herd and therefore not Trojan dams),
- the maximum number of Trojan dams (all dams identified in 3, 4a, b, c and d), based on the assumption that all dams entering the birth herd between days 30 and 120 of gestation became infected in a source herd and therefore were Trojan dams
- ‘study Trojan dams’, for the purposes of this study which were defined as 3, 4a, c and d dams. Therefore, these dams each produced a ‘study Trojan birth’ during the study period.

2.4. Data analyses

2.4.1. Descriptive analyses

Data analyses were conducted using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA), SAS 9.4 (SAS Institute Inc., 2008) and STATA 14 (StataCorp LP, College Station, TX, USA). In the initial descriptive analyses, we report on the minimum and maximum number of Trojan dams. In all subsequent descriptive, and all multivariable, analyses, we solely focus on study Trojan dams.

The number of BVD+ calves born and the number of birth events (including twin births) with BVD+ calves in Ireland were calculated for 2013, 2014, 2015 and in total. We calculated the percentage of all BVD+ birth events that were attributable to Trojan dams by year and by a range of independent variables relating to the birth herd (region, herd type, herd size), the dam (parity, whether the dam calved in the herd of origin or not) and birth event (month of birth). We then calculated the number of herds with at least one BVD+ birth and the percentage of these herds with all BVD+ births attributable to a Trojan dam, by year and herd type, consistent with these therefore representing the only BVD+ births in these herds in that year. Data on source herds and prior movements were not available for 11 Trojan dams which had been imported from outside the Republic of Ireland (ROI). For all other dams, we calculated the number of movements during pregnancy and the percentage of these dams with at least one movement via a market whilst pregnant, by herd type and year of birth.

2.4.2. Multivariable analyses

2.4.2.1. Birth herds. A logistic regression model was developed using SAS version 9.4 to model the probability of a dam to a BVD+ calf being a Trojan, as opposed to a non-Trojan dam, in 2015. The unit of interest

Table 1

The number of BVD+ calves born, the number of birth events with at least one BVD+ calf, and the number of birth events with more than one BVD+ calf in Ireland during 2013, 2014 and 2015, and in total. The total number of birth events corresponds to the total number of dams in the study.

	2013	2014	2015	Total
Number of BVD+ calves born	14,187	10,040	7445	31,672
Number of birth events with at least one BVD+ calf	13,929	9683	7372	30,984
Number of those birth events with more than one BVD+ calf ^a	254	350	73	677

^a Number of birth events where multiple BVD+ calves were born. In 2013, there were 250 twins and 4 triplets. In 2014, there were 343 twins and 7 triplets. In 2015, all multiple births were twins.

was the dam and the dataset included one record for every dam of a BVD+ calf born in 2015. The outcome was whether the dam was a Trojan or not. A Generalized Estimating Equation (GEE) method was used to account for the clustering of dams within the same birth herds (Dohoo et al., 2009). This method was considered more suitable than using mixed models due to the sparseness of multiple dams within many of the birth herds (McNeish et al., 2014). The choice of correlation structure (either independence or unstructured) within the GEE model was based on the quasi-information criteria (QIC) (Dohoo et al., 2009).

The following independent variables relating to the birth herd (the herd in which the BVD+ calf was born in 2015) or the dam of the BVD+ calf were considered in the model: region (seven regions), herd type (dairy/beef/dual), herd size (number of animals in the herd on 1st Jan, 2015), dam parity (1st, 2nd, 3rd, ≥4th), and season of birth. Initially each risk factor was tested using a univariable chi squared test. Risk factors that were significant at the univariable stage ($p < 0.20$) were used to build a multivariable model. A backward selection procedure was used to eliminate terms from the multivariable model based on a generalised score test ($p > 0.05$). Collinearity was assessed using Spearman's correlation coefficient ($rsp > 0.7$) or chi-square tests ($p < 0.05$). The appropriate format or transformation of continuous variables was based on a plot of the log odds of the outcome and the variable. Transforming variables to categorical formats was based on the quintiles of the relevant distribution. Two-way interactions between herd type and risk factors of herd size, region, season of birth, and parity were each tested in the initial model. An assessment of the goodness-of-fit of the model was obtained using plots of delta-betas and leverage values clustered at the birth herd level.

2.4.2.2. Source herds. A logistic regression model was developed to identify risk factors for a BVD+ herd selling a Trojan dam. The study population included all herds with at least one BVD+ animal present in the herd during 2014 (these were potential 'source herds'). The outcome was whether the herd sold one (or more) animals in 2014 that was subsequently identified as a Trojan dam. These Trojan dams each had a calf within 252 days of moving from the source herd (282 days gestation minus 30 days prior to the start of the window of susceptibility), with the birth occurring either in 2014 or 2015. The risk factors considered in the model, each relating to herds with BVD+ animals in 2014, included:

- region (seven regions)
- herd size (number of animals in the herd on 1st Jan, 2014)
- number of calves born in 2014
- herd type (dairy/beef/dual)
- season of calving (Spring/Autumn/both)
- number of female cattle > 12 m of age sold to another herd in 2014 in total and by age groups (12–24 m, 24–36 m and > 36 m)
- number of pregnant females > 12 m of age sold to another herd in

2014 in total (with a calving date within 8 months of being sold) and by age groups (12–24 m, 24–36 m and > 36 m sold)

- number of BVD+ animals in the herd in 2014
- duration of positivity (total number of days that one or more BVD+ animal was present in the herd in 2014).

The appropriate format of continuous variables was assessed by plotting the variable against the log-odds of a case. If the relationship did not appear to be linear (and a transformation was not appropriate), the variable was treated as categorical (based on the quintiles of the continuous variable). Each risk factor was initially tested using a univariable logistic regression model, with those that were significant at this stage ($p \leq 0.20$) being considered for inclusion in the multivariable model. A backward selection procedure was used to eliminate terms from the multivariable model based on a likelihood ratio test ($p > 0.05$). Collinearity was assessed using Spearman's correlation coefficient ($rsp > 0.7$) or chi-square tests ($p < 0.05$). The choice of variable to include in the model when two or more were correlated was based on the AIC. Interaction terms between herd type and region, season and herd size were each tested in the model along with an interaction between number of BVD+ in the herd and the duration of positivity. An assessment of the goodness-of-fit of the model was obtained using a Hosmer-Lemeshow test and plots of delta-betas and leverage values.

3. Results

During 2013–15, 31,672 BVD+ calves were born in 14,473 farms in Ireland, including 14,187 calves in 2013, 10,040 in 2014 and 7445 in 2015 (Table 1). There were 677 multiple birth events of BVD+ calves during this period, including 666 twin births and 11 triplet births. Of these 31,672 BVD+ calves, 68% were BVDPI and 32% were BVDPOS.

Of the 30,984 dams that gave birth to BVD+ calves during the study period, 1562 dams were BVD+ themselves, therefore a maximum of 29,422 (95.0%) dams, giving birth in 13,577 herds, could potentially have been Trojan dams. Of these, 2953 (10.0%) dams entered the birth herd while pregnant, including 322 before day 30 of gestation, 925 between days 30 and 120 of gestation, and 1706 after day 120 of gestation (Table 2). The minimum, and maximum number of Trojan dams was 1706 (5.8% of the 29,422 dams), 2631 (8.9%), respectively. Further, there were 2526 (8.6%) study Trojan births, including 938 (7.1% of all BVD+ birth events to non-BVD+ dams), 841 (9.2%) and 747 (10.6%) during 2013, 2014 and 2015, respectively (Table 3).

Table 2

The number (%) of dams of BVD+ calves born in Ireland during 2013–15 that were not themselves BVD+, by Trojan status.^a

Trojan status	Present in birth herd for the full duration of gestation Q1	Moved into the birth herd before day 30 of gestation Q2	Moved into the birth herd after day 120 of gestation Q3	Moved into the birth herd between day 30 and 120 of gestation				Total
				Q4 (Based on potential sources of exposure for the dam to BVDv)				
				a	b	c	d	
Trojan	–	–	1706	720	–	77	23	2526
Non-Trojan	26,469	322	–	–	105	–	–	26,896
Total	26,469	322	1706	720	105	77	23	29,422 ^b

^a Methods to assign Trojan dam status are outlined in the Materials and Methods.

^b During 2013–15, there were 29,422 BVD+ birth events to non-BVD+ dams. Although 30,984 dams gave birth to BVD+ calves during the study period, 1562 of these dams were BVD+.

Table 3

The total number of BVD + birth events to non-BVD + dams, and the percentage of these attributable to Trojan dams, by year and by variable relating to the birth herd (region, herd type, herd size), the dam (parity, whether dam calved in the herd of origin) and birth event (season of birth). Chi square tests were used to test the association between % Trojan births and each of these independent variables. The methodology to determine Trojan status is described in the Materials and Methods, and calculations were restricted to the 2526 most-likely Trojan dams.

	2013			2014			2015			2013–15		
	Number of BVD + birth events	Number (%) of Trojan births	P-value ^a	Number of BVD + birth events	Number (%) of Trojan births	P-value ^a	Number of BVD + birth events	Number (%) of Trojan births	P-value ^a	Number of BVD + birth events	Number (%) Trojan births	P-value ^b
Total	13,173	938 (7.1)		9185	841 (9.2)		7064	747 (10.6)		29,422	2526 (8.6)	< 0.001
Region			< 0.001			0.009			0.002			< 0.001
Border	2159	182 (8.4)		1411	130 (9.2)		1101	117 (10.6)		4671	429 (9.2)	
West	2151	166 (7.7)		1399	124 (8.9)		978	118 (12.1)		4528	408 (9.0)	
Midlands	1367	122 (8.9)		992	119 (12.0)		658	88 (13.4)		3017	329 (10.9)	
Midlands-East	892	68 (7.6)		703	78 (11.1)		699	61 (8.7)		2294	207 (9.0)	
Southeast	2641	150 (5.7)		1950	165 (8.5)		1538	178 (11.6)		6129	493 (8.0)	
Southwest	2656	139 (5.2)		1712	144 (8.4)		1270	102 (8.0)		5638	385 (6.8)	
Midwest	1307	111 (8.5)		1018	81 (8.0)		820	83 (10.1)		3145	275 (8.7)	
Herd type			< 0.001			< 0.001			0.001			< 0.001
Dairy	5559	306 (5.5)		3909	292 (7.5)		2999	272 (9.1)		12,467	870 (7.0)	
Beef	6562	528 (8.0)		4513	457 (10.1)		3346	380 (11.4)		14,421	1365 (9.5)	
Dual purpose	1052	104 (9.9)		763	92 (12.1)		719	95 (13.2)		2534	291 (11.5)	
Herd size ^c			< 0.001			< 0.001			< 0.001			< 0.001
< 40	2510	10.4%		1665	13.7%		1227	15.7%		5402	12.6%	
41–80	2877	7.1%		1882	9.1%		1446	10.0%		6205	8.4%	
81–120	2418	6.1%		1507	6.4%		1134	9.1%		5059	6.9%	
121–200	2872	5.5%		2035	7.1%		1538	7.6%		6445	6.5%	
> 200	2496	6.7%		2096	9.5%		1719	11.1%		6311	8.8%	
Dam parity ^c			< 0.001			< 0.001			< 0.001			< 0.001
1	4994	617 (12.4)		3625	554 (15.3)		2850	18.0	512 (18.0)	11,469	1683 (14.7)	
2	2835	115 (4.1)		1972	120 (6.1)		1328	7.6	101 (7.6)	6135	336 (5.5)	
3	1832	85 (4.6)		1243	62 (5.0)		1005	4.7%	47 (4.7)	4080	194 (4.8)	
4+	3512	121 (3.4)		2345	105 (4.5)		1881	4.6%	87 (4.6)	7738	313 (4.0)	
Dam calved in herd of origin ^d			< 0.001			< 0.001			< 0.001			< 0.001
No	4708	788 (16.7)		3392	655 (19.3)		2750	640 (23.3)		10,850	2083 (19.2)	
Yes	8465 ^e	150 (1.8%)		5793	186 (3.2)		4314	107 (2.5)		18,572	443 (2.4)	
Birth season			< 0.001			< 0.001			< 0.001			< 0.001
Spring	7205	418 (5.8)		4772	332 (7.0)		3766	330 (8.8)		15,743	1080 (6.9)	
Summer	2606	257 (9.9)		1889	226 (12.0)		1439	205 (14.2)		5934	688 (11.6)	
Autumn	1409	127 (9.0)		1208	141 (11.7)		880	107 (12.2)		3497	375 (10.7)	
Winter	1953	136 (7.0)		1316	142 (10.8)		979	105 (10.7)		4248	383 (9.0)	

^a The univariable association between each risk factor and the likelihood of a BVD + birth being to a Trojan dam in the year of interest. The chi squared test was calculated separately for each year of the study period.

^b The association between each risk factor and the likelihood of a BVD + birth being to a Trojan dam in the full study period.

^c During the year of the BVD + birth.

^d Whether dam calved in the same herd in which she was born, noting that this does not consider movements between dam's birth and birth of the PI calf.

^e In 2013, 8465 of the BVD + births occurred in herds in which the dam had herself been born. Of these births, 1.8% were attributed to Trojan dams.

During 2013–15, there were 13,577 herds with at least one BVD + birth to a non-BVD + dam (Table 4). In 2013, there was at least one Trojan birth in 9.9% of the herds with at least one BVD + birth to a non-BVD + dam, increasing to 11.8% in 2014 and 13.3% in 2015. In 8.3% of the 13,577 herds with at least one BVD + birth to a non-BVD + dam during 2013–15, all of these births could be attributed to Trojan dam(s). Separately during 2013, 2014 and 2015, these percentages were 7.8%, 9.2% and 10.7%, respectively.

Full movement records were available for 2513 (99.5%) of the 2526 Trojan dams (the remaining 13 dams were imported to the birth herd from outside Ireland and movement history was not available). Of these 2513 dams, 1754 (69.8%) had moved once, 610 (24.3%) had moved twice and 149 (5.9%) more than twice during the pregnancy of interest (Table 5).

There was a significant univariable association between each of the

independent variables and whether a BVD + birth from a non-BVD + dam was attributable to a Trojan dam or not (Table 3). The percentage of BVD + births from non-BVD + dams attributable to Trojan dams was highest in the Midlands and lowest in the South-West, though there were yearly variations in this trend. Trojan births were most frequent in dual purpose herds and least frequent in dairy herds. The highest proportion of BVD + births were in spring, while the highest percentage of these due to a Trojan dam was in summer in each year of the study. BVD + births from non-BVD + dams attributable to Trojan dams were highest in herds with less than 20 animals. Herds with more than 260 animals had the next highest proportion of Trojan births. Trojan births were more common among first parity dams compared with other dam parities.

In 2015, there were 7064 dams of BVD + calves in 4023 birth herds, including 2778 (69%) birth herds with just a single dam giving birth to

Table 4
The number of herds with at least one BVD+ birth to a non-BVD+ dam, and of these, the number (%) of herds with at least one or only Trojan dams, by year and herd type.^a

	Dairy	Beef	Dual	Total
Number of herds with at least one BVD+ birth to a non-BVD+ dam				
2013	2782	4306	567	7655
2014	1897	2974	423	5294
2015	1483	2189	351	4023
2013–2015 ^b	4711	7860	1006	13,577
Of these:				
The number (%) of herds with at least one Trojan birth				
2013	206 (7.4)	460 (10.7)	89 (15.7)	755 (9.9)
2014	158 (8.3)	393 (13.2)	73 (17.3)	624 (11.8)
2015	138 (9.3)	330 (15.1)	67 (19.1)	535 (13.3)
2013–2015 ^b	443 (9.4)	1151 (14.6)	200 (19.9)	1794 (13.2)
The number (%) of herds with only Trojan births				
2013	148 (5.3)	388 (9.0)	62 (10.9)	598 (7.8)
2014	118 (6.2)	321 (10.8)	46 (10.9)	485 (9.2)
2015	93 (6.3)	288 (13.2)	50 (14.2)	431 (10.7)
2013–2015 ^b	234 (5.0)	774 (9.8)	115 (11.4)	1123 (8.3)

^a These calculations are restricted to the 2526 study Trojan dams.

^b The number of herds for 2013–2015 will be less than the sum of the number of herds in individual years because some herds had BVD+ calves in more than one year.

Table 5
The number (%) of Trojan dams that moved once, twice or more than twice during pregnancy, by herd type and year of birth.

	Number (%) of Trojan dams ^a			Total	Number (%) with at least one movement via a market
	1 movement whilst pregnant	2 movements whilst pregnant	> 2 movements whilst pregnant		
Total	1754 (69.8)	610 (24.3)	149 (5.9)	2513	981 (39.0)
Herd type					
Dairy	616 (71.1)	207 (23.9)	43 (5.0)	866	162 (18.7)
Beef	930 (68.4)	340 (25.0)	90 (6.6)	1360	693 (51.0)
Dual	208 (72.5)	63 (22.0)	16 (5.6)	287	126 (43.9)
Birth year					
2013	688 (73.9)	198 (21.3)	45 (4.8)	931	366 (39.3)
2014	561 (67.0)	230 (27.5)	46 (5.5)	837	325 (38.8)
2015	505 (67.8)	182 (24.4)	58 (7.8)	745	290 (38.9)

^a During 2013–15, there were 29,422 BVD+ birth events to non-BVD+ dams of which 2526 were attributed to Trojan dams. Movement data were available for 2513 (99.5%) of these latter dams; the remaining 13 were imported to the birth herd from outside Ireland and full movement history was not available.

a BVD+ calf in 2015. Of the 7064 dams, 747 (10.6%) were Trojan dams in 535 birth herds. Sixteen dams (all of which were Trojan dams) from 13 birth herds were not included in the modelling due to missing herd-size data. All variables were significant in the univariable model ($p < 0.002$).

The final multivariable GEE model included dam parity, herd size (as a categorical variable) and an interaction between herd type and season of birth (Table 6). A model using independent correlation structure was the only one that would converge. A dam of a BVD+ calf was more likely to be a Trojan if she was a first parity dam, in a small herd (< 46 animals), or calved during summer for dairy herds or during winter for beef herds (no significant difference associated with season was detected for dual herds). The only significant difference by herd-type was for winter births when the dam was more likely to be a Trojan in beef herds.

Examination of the delta-beta and leverage values indicated an outlier herd. This herd was a dealer herd with 41 dams of BVD+ calves, 38 of which were Trojan dams. When this herd was excluded from the model, the difference between the largest herd size category and the referent also became significant with the OR reducing from 0.72 to 0.57 (95% CI: 0.36–0.91). However, there were no other major differences to

the model when this herd was excluded.

A total of 8252 herds in 2014 had one or more BVD+ animal. Of these, 189 (2.3%) of these herds sold a Trojan dam in 2014. In the initial univariable analysis, all risk factors were significant ($p < 0.02$) with the exception of 'region' ($p = 0.577$) and the 'duration of positivity' ($p = 0.425$). Variables related to the number of females sold or the number of pregnant females sold were correlated, therefore the variable 'number of pregnant females > 12 m sold' was selected for inclusion in the multivariable model based on the AIC from the univariable models.

The final multivariable model included the variables: 'number of pregnant females > 12 m sold' and 'number of BVD+ animals in the herd in 2014' (Table 7). All interactions tested within the multivariable model were not significant ($p > 0.05$). The Hosmer-Lemeshow test ($p = 0.493$) and plots of delta-betas and leverage indicated no significant lack of fit. Since the majority of source herds (4883, 59%) had only 1 BVD+ animal, only 3 groups were created for the variable 'number of BVD+ animals in the herd in 2014' (Table 7). Similarly for the variable 'number of pregnant females > 12 m sold' the majority of herds (6112, 74%) did not sell any pregnant females aged over 12m. However, since by definition these herds did not sell any Trojan dams, these herds were combined with herds selling 1 pregnant female aged over 12 m (780 herds). The odds of selling a Trojan dam in herds selling more than 1 pregnant female aged over 12m was 37 times higher compared to herds selling 0 or 1 such animals. Herds with > 2 BVD+ in the herd in 2014 were 1.6 times more likely to sell a Trojan dam compared to herds with just 1 BVD+ in 2014.

4. Discussion

While many authors have highlighted the role of Trojan dams in the epidemiology of BVD (Houe, 1999; Hult et al., 2005; Presi et al., 2011), the lack of detailed animal-level data has limited the opportunity to quantify their role at a national level. In 2001, Alban et al. (2001) considered the role of Trojan dams in the larger context of BVD transmission in Denmark. In the current study, drawing on animal-level data generated in Ireland, we present a detailed analysis of the contribution of Trojan dams to the epidemiology of BVD in Ireland in the context of the national eradication programme.

This study has highlighted the important role played by Trojan dams in the spread of BVDv in Ireland. During 2013–15, 8.6% of all BVD+ birth events could be reasonably attributed to Trojan dams (Table 3). In herds with at least one BVD+ birth to a non-BVD+ dam each year, at least one of these was attributed to a Trojan dam in 9.9% of herds in 2013, 11.8% in 2014 and 13.3% in 2015. More importantly, all BVD+ births could be attributed to Trojan dams in 7.8%, 9.2% and 10.7% of herds with at least one BVD+ birth to a non-BVD+ dam during 2013, 2014 and 2015, respectively (Table 4). At the time of study, none of these herds had achieved Negative Herd Status (NHS) (certifying the absence of BVDv on the farm) given that one of the prerequisites for this status is a minimum of three years of tissue tag testing of calves. Therefore, while none of the herds mentioned were eligible for this status, attributing all BVD+ births to a Trojan dam is based on best available knowledge that there were no other BVD+ animals present to account for BVDv presence. In earlier work, Hult and Lindberg (2005) suggested that the relative importance of Trojan dams would be expected to increase during a control programme, as direct contacts between BVD+ and naive animals in different herds are reduced, and secondary routes of transmission therefore become more important. In the current study, we confirm the emergence of this pattern at the herd level, with year-on-year increase in the percentage with BVD+ births to non-BVD+ dams attributable to Trojan births, both in total and for each herd type (Table 4). These findings highlight the impact of Trojan dams in the spread of BVDv to farms that otherwise would have had fewer or no BVD+ births in the relevant year. As demonstrated in the current study, Trojan births increase the number of

Table 6
Parameter estimates from the multivariable logistic GEE regression model of the Trojan status of dams of BVD+ calves in 2015.

Variable	Category	Estimate	S.E.	O.R.	95% C.I.		p ^a	p ^b
					Upper	Lower		
Parity of dam	1st	Referent		1.00				
	2nd	-1.04	0.13	0.35	0.27	0.46	< 0.001	< 0.001
	3rd	-1.61	0.18	0.20	0.14	0.28	< 0.001	
	≥4th	-1.61	0.14	0.20	0.15	0.26	< 0.001	
Herd size	1–45	Referent		1.00				< 0.001
	46–86	-0.68	0.15	0.50	0.37	0.68	< 0.001	
	87–142	-0.50	0.18	0.61	0.43	0.86	0.005	
	143–228	-1.05	0.23	0.35	0.22	0.54	< 0.001	
	> 228	-0.33	0.29	0.72	0.41	1.27	0.256	
Herd-type x Season of birth interaction								
Herd-type (Spring)	Dairy	Referent		1.00				
	Beef	-0.08	0.24	0.93	0.58	1.48	0.747	
	Dual	0.19	0.38	1.21	0.58	2.52	0.619	
Herd-type (Summer)	Dairy	Referent		1.00				
	Beef	-0.25	0.31	0.78	0.42	1.42	0.416	
	Dual	0.52	0.38	1.68	0.80	3.54	0.172	
Herd-type (Autumn)	Dairy	Referent		1.00				
	Beef	-0.10	0.38	0.90	0.43	1.92	0.794	
	Dual	0.46	0.50	1.59	0.60	4.21	0.350	
Herd-type (Winter)	Dairy	Referent		1.00				
	Beef	0.75	0.30	2.12	1.19	3.77	0.011	
	Dual	0.04	0.55	1.04	0.36	3.04	0.936	
Season of birth (Dairy herds)	Spring	Referent		1.00				
	Summer	0.69	0.34	1.99	1.01	3.90	0.046	
	Autumn	0.26	0.41	1.30	0.58	2.92	0.520	
	Winter	-0.29	0.26	0.75	0.45	1.24	0.259	
Season of birth (Beef herds)	Spring	Referent		1.00				
	Summer	-0.17	0.38	0.84	0.40	1.78	0.648	
	Autumn	-0.02	0.45	0.98	0.40	2.37	0.959	
	Winter	0.83	0.33	2.29	1.20	4.34	0.012	
Season of birth (Dual herds)	Spring	Referent		1.00				
	Summer	0.33	0.54	1.39	0.49	3.98	0.535	
	Autumn	0.28	0.64	1.32	0.38	4.58	0.662	
	Winter	-0.14	0.59	0.87	0.27	2.77	0.809	

^a Z-test.

^b Generalised Score test.

^c P-value for the interaction term: Herd-type x Season of birth.

Table 7
Parameter estimates from the logistic regression model of BVD+ herds in 2014 that sold a Trojan dam.

Variable		Estimate	S.E.	OR	95% confidence interval		P-value ^a	P-value ^b
					Lower	Upper		
Intercept		-5.77	0.21					
Number of pregnant females > 12 m sold in 2014	0–1	0.00		1.00				< 0.001
	> 1	3.61	0.22	36.98	24.17	56.60	< 0.001	
Number of BVD+ animals in herd in 2014	1	0.00		1.00				0.032
	2	0.23	0.19	1.26	0.86	1.84	0.239	
	> 2	0.48	0.18	1.62	1.13	2.32	0.008	

^a Z-test.

^b Generalised Score test.

herds in which infection is present. Further, they can have a direct impact on the birth herd through the generation of further BVD+ calves in the subsequent season (Graham et al., 2014a), and also place neighbouring herds at greater risk of infection subsequently, particularly if the BVD+ calf is not removed promptly (Graham et al., 2016). Collectively, this can be expected to delay progress to eradication. At the animal level, an increase in the percentage of BVD+ birth events attributable to Trojan dams was observed in each year of the study (Table 3), consistent with that seen at herd level.

A number of predictors for herds having BVD+ births attributable to Trojan dams were identified. In all years, significant differences in the percentage of Trojan births were observed between herd types,

being consistently lowest in dairy herds, when compared to beef and dual purpose herds (Table 3). The purchase of replacement heifers for enterprises with a beef component (dual purpose or beef herds) is relatively common in Ireland, with 40% of replacements being purchased rather than homebred (Murray et al., 2012), whereas dairy herds predominantly breed their own replacement heifers. In addition, pregnant animals entering dairy enterprises are the least likely to have moved through a market (Table 5), reducing the number of direct and indirect animal contacts and the likelihood that these animals are exposed to BVDv from these sources. While interactions between herd type and region were not significant in multivariable analysis, over the three years of this study, the South-East and South-West had the highest

numbers of BVD+ births, while the greatest proportion of BVD+ births attributable to Trojan dams was in the Midlands and Borders (Table 3), while this proportion was lowest in the South-West. This may be related to the high concentration of dairy herds in the South-West region, as dairy herds are associated with a lower proportion of Trojan births.

In the birth herd model (Table 6), we focused on births in 2015, as the most recent data available. Information on the BVD status of each herd and individual animals was most complete in this year. Further, using a single year allowed us to reduce the number of hierarchical levels in the model. BVD+ births were found to be more likely to be due to a Trojan dam in a beef herd than in a dairy herd, but only in winter. This could reflect the more common practice in beef, compared to dairy, herds of purchasing in-calf replacement animals. Beef herds will tend to breed for terminal traits such as growth and carcass conformation, rather than maternal traits. However, this does not explain why there should be more Trojans in winter only. A more detailed analysis of purchase patterns in beef and dairy herds is beyond the scope of the current study. Multivariable analysis also highlighted the risk associated with buying in-calf maiden heifers. The risk of a Trojan birth was highest in in-calf maiden heifers with the risk decreasing with dam age, presumably because older dams are more likely to have come in contact with, and to have developed immunity to, BVDv before this pregnancy. The odds of having a Trojan birth were also greatest in smaller herds (1–45 animals) and least in medium sized herds (143–228 animals) (Table 6).

BVD+ herds form only a proportion of all possible source herds. However, only BVD+ source herds were considered in this study since these will be the herds to which any future movement restrictions can most readily be applied. The majority (74%) of BVD+ herds in 2014 did not sell any pregnant females aged over 12 months. The herds with the highest odds of selling a Trojan dam were those selling more than one pregnant female and with a higher infection pressure (> 2 BVD+ present in the herd).

There is a need to evaluate strategies used to limit the impact of Trojan dams in the spread of BVDv in the context of the national eradication programme, with respect to both their effectiveness (the proportion of Trojan dams that could be prevented from moving from source herds) and proportionality (the impact on trade from source herds). One option is a prohibition on all females over a certain age leaving herds in which BVD+ calves are born for a defined period following the removal of the last BVD+ animal. This would reduce the risk of animals with an unknown pregnancy status being traded and potentially going on to become Trojan dams. However, in the absence of further knowledge of the patterns of trade from these herds, particularly the total numbers of females sold and the number of these that are pregnant, such a measure could be disproportionate. In this context, it is encouraging to note that only 26% of BVD+ source herds in 2014 sold pregnant females over 12 months of age. Alternatively, restrictions on movements from herds with BVD+ births could be limited to seropositive pregnant animals only, although this approach would incur additional costs in terms of pregnancy diagnosis and serological testing.

There were a number of challenges encountered whilst conducting this study, relating to both the availability of data and the biology of BVDv, which presented difficulties when defining the Trojan status of the dam of each BVD+ calf. It was necessary to define a specific time period for the window of susceptibility, with 30–120 days of gestation being used here. While these limits are generally accepted, it is acknowledged that exposure before or after these time points could result in BVD+ births (Grooms, 2004; Burciaga-Robles et al., 2010). Dams producing BVD+ calves in this study were considered non-Trojan if they entered the herd before day 30 of gestation, based on the published literature (Rufenacht et al., 2000). In our study, 3.1% of all non-BVD+ dams giving birth to a BVD+ calf entered the birth herd during the WOS (Table 2). For these animals, Trojan status was assigned based on the best available information regarding the presence or absence of identifiable source(s) of BVDv infection in the source and birth herds, as

described in Section 2.3. Under the national eradication programme, which became compulsory in 2013, there is an assigned status for each animal in every breeding herd in the country. 2013 was the first year that all calves were tested for BVDv nationally, giving a direct and indirect status, respectively, to each calf and its dam, however, older, untested animals were classified as unknown, potentially being positive or negative for BVDv. The number of animals with a direct or indirect known BVD status has been increasing since 2013. Further, older animals with an unknown BVD status are progressively being culled from the national herd. Over 2 million calves were tagged in each year of the programme, and a BVD status was available for an estimated 98% of the national herd by December 2015 (unpublished data). Therefore, the BVD status of a greater percentage of animals within herds was known in 2015 when compared to 2014, and particularly 2013, reducing the possibility that unidentified BVD+ animals were present in either source or birth herds. During 2013 in particular, both source and birth herds typically contained animals whose status was unknown. Given that some of these animals of unknown status could have been BVD+, it is possible that both source and birth herds could have been misclassified as negative. The presence of animals of unknown status has previously been shown to be a herd-level risk factor for the birth of BVD+ calves (Graham et al., 2016). It is also recognised that dams in source herds may have acquired infection from outside the herd, as opposed to from a BVD+ animal within the herd or during the trade process.

With this in mind, a minimum and maximum number of Trojans was calculated, reflecting the uncertainty associated with these estimates. In the Irish context during 2013–15, the minimum and maximum number of Trojan dams was 1706 (5.8% of the 29,422 dams) and 2631 (8.9%) respectively. The study Trojan dams (2526 being 8.6% of 29,422 birth events to dams that were themselves not BVD+) was used for all further calculations.

In conclusion, the current study has for the first time used data generated from a national tissue tag-based BVDv eradication programme to quantify the proportion of BVD+ births attributable to Trojan dams and also identified risk factors for both herds generating Trojan dams and those introducing them. The findings clearly demonstrate the role of Trojan dams as a source of BVD+ births, accounting for all BVD+ births in 8.3% of herds that had one or more BVD+ births to non-BVD+ dams in the study period (Table 4). Addressing this route of transmission will be critical to achieving eradication in as short a time period as possible and in enabling herds that are now free of infection to continue to introduce animals without introducing BVDv. Further studies are currently underway to evaluate both the effectiveness and proportionality of a range of strategies to minimize the impact of Trojan dams. These studies will provide an evidence base to inform any necessary policy changes, including revisions to the legislation governing the eradication programme. Currently in the Irish eradication programme, all calves are tagged and tested for BVDv at birth. If an option to use serological monitoring were to be introduced in advance of eradication being achieved, it would further increase the importance of measures to prevent movement of Trojan dams, since the birth of Trojan calves in BVDv-free herds could go undetected until screening was carried out when the birth cohort was at least 9 months of age, with the potential to create multiple further PI births and increase the time to eradication (Graham et al., 2014a; Thulke et al., 2017).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

FR designed the study, conducted the statistical analyses and drafted the manuscript. SM coordinated the research. JT and TC advised statistical analysis. TC conducted regression analyses. POS provided the data for analysis. DG oversaw the study design. All authors

contributed to ongoing manuscript review and approved the final document.

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