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# Enhancing bovine abortion surveillance: A learning experience

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# ABSTRACT

Abortions and perinatal mortalities (APM) substantially affect cattle industry efficiency. Various infectious and noninfectious factors have been associated with bovine APM worldwide. Infections are often considered pivotal due to their abortifacient potential, leading laboratories to primarily investigate relevant infectious agents for APM cases. Some infectious causes, such as Brucella abortus, have also a zoonotic impact, necessitating monitoring for both animal and human health. However, underreporting of bovine APM is a global issue, affecting early detection of infectious and zoonotic causes. Previous studies identified factors influencing case submission, but regional characteristics may affect results. In Belgium, farmers are obliged to report cases of APM within the context of a national brucellosis monitoring program. The inclusion criteria for this monitoring program cover abortions (gestation length of 42–260 d) and perinatal mortalities of (pre) mature calves following a gestation length of more than 260 d, which were stillborn or died within 48 h after birth. The objective of the present study was to describe the evolution in submission of APM cases within a mandatory abortion monitoring program in relation to subsidized initiatives in the northern part of Belgium. Based on the proportion of APM submissions versus the proportion of bovine reproductive females, an APM proportion  $(APM_{PR})$  was calculated, and factors at both animal and herd level that may influence this APM<sub>PR</sub> were explored by using linear models. This evaluation revealed that the  $APM_{PR}$  increased with the introduction of an extensive analytical panel of abortifacient agents and a free on-farm sample collection from 0.44% to 0.94%. Additionally, an increase of the  $APM_{PR}$  was associated with an outbreak of an emerging abortifacient pathogen (Schmallenberg virus; 1.23%), and the introduction of a mandatory eradica-

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tion program for bovine viral diarrhea virus (BVDv; 1.20%). The APM<sub>PR</sub> was higher in beef compared with dairy cattle, and it was higher in winter compared with fall, spring, and summer. Smaller herds categorized in the first quartile had a higher  $APM_{PR}$  compared with larger herds. Herds that submitted an APM in the previous year had a higher  $APM_{PR}$  in the next year compared with herds without an APM submission. Finally, herds for which there was evidence of the presence of BVDv had a higher APM<sub>PR</sub> compared with herds without evidence of the presence of BVDv. In conclusion, the number of APM submissions increased after the introduction of a free on-farm sample collection and an extensive pathogen screening panel. Production type (beef), season (winter), smaller herd size, previous APM, and presence of BVDv seemed to have a positive effect on  $APM_{PR}$ . However, even under mandatory circumstances, APM still seems to be underreported, since the  $APM_{PR}$  was lower than the expected minimal rate of 2%. Therefore, further research is necessary to identify the drivers that convince farmers to submit APM cases to improve submission rates and ensure an efficient monitoring program for APM and eventually associated zoonotic pathogens. **Key words:** cattle, abortion, perinatal mortality,

disease monitoring

# INTRODUCTION

Abortions and perinatal mortalities (**APM**) have a major economic impact on reproduction and production efficiency in the cattle industry. The term "abortion" typically refers to pregnancy loss in the fetal stage, which spans from 42 to 260 d of gestation, including early fetal loss (**EFL**) occurring between 45 and 60 d of gestation, and late fetal loss (**LFL**) occurring between 60 and 260 d of gestation. In dairy cattle, the expected incidence of EFL is around 7%, whereas LFL ranges from 1% to 3% (Wiltbank et al., 2016; Albaaj et al., 2023). However, for beef cattle, specific abortion thresholds have not been determined. Perinatal mortality has been defined as the loss of a nonviable

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fetus beyond 260 d of gestation, and deaths of full-term calves up to 48 h of age (Mee, 2013). In dairy cattle, the perinatal mortality rate ranges from 2.4% to 9.7%, with a median of 6.6% (Cuttance and Laven, 2019), but this rate also remains unknown for beef cattle.

Many infectious and noninfectious factors have been described to be involved in bovine APM in different regions and countries worldwide (Mee, 2013; Clothier and Anderson, 2016; Wolf-Jäckel et al., 2020; Van Loo et al., 2021). While noninfectious causes are important, particularly in cases of perinatal mortality (Jawor et al., 2017), infections are generally considered to be more relevant due to their substantial potential to cause abortions. As a result, in most laboratories, the primary emphasis is placed on investigating the infectious agents that are most relevant and prevalent in a particular region when studying cases of APM. Depending on the region, numerous infectious agents may be involved in bovine APM, including bacteria (e.g., Brucella abortus, Pajaroellobacter abortibovis, and Trueperella pyogenes), yeasts or molds, viruses (e.g., bovine herpes virus type 1, bovine viral diarrhea virus, and Schmallenberg virus), and parasites (e.g., *Neospora caninum*). Several infectious causes of APM have also a zoonotic impact (e.g., Brucella abortus, Salmonella spp., Coxiella burnetii, and Chlamydia spp.), which makes monitoring of infectious causes of APM crucial for both animal and human health. As for all diseases, the monitoring of APM relies on the notification and reporting of suspected cases (Bronner et al., 2013, 2014). However, underreporting of detected bovine APM is a major issue worldwide, although it is mandatory to report in many countries. For instance, in Canada and France it was reported that less than 40% of farmers were motivated to submit cases of APM for analysis (Bronner et al., 2014; Denis-Robichaud et al., 2019), whereas in New Zealand, it was only 5.5% (Thobokwe and Heuer, 2004). This underreporting may hamper the early detection of zoonotic and other infectious causes of APM. Previous studies have identified several factors that influence the decision of farmers and veterinarians to submit APM cases, such as the perceived risk of causes of APM, the adopted definition of APM, the costs and benefits of case analysis, and practical considerations related to submitting a case (Bronner et al., 2014; Clothier et al., 2020). However, it is important to note that the results of these studies may be affected by regional characteristics. Therefore, the objectives of the present study were (1) to evaluate trends in APM submissions and (2) to identify factors that may influence the number of submitted APM cases in the northern region of Belgium (Flanders).

## MATERIALS AND METHODS

## Background

In Belgium, the prevalence of bovine brucellosis has substantially decreased over the years, leading to the country being declared officially free of the disease by the European Union in 2003 (Commission Decision 2003/467/EC, 2003). However, surveillance remains essential, as demonstrated by the re-emergence of *Brucella* outbreaks in Belgium between 2010 and 2013. From 1978 onward, one of the main pillars of bovine brucellosis surveillance in Belgium has been the obligatory reporting of cases of APM by farmers to their corresponding regional sanitary veterinarian, responsible for the epidemiological surveillance of the herd. The inclusion criteria for this APM monitoring program cover abortions (gestation length of 42–260 d) and perinatal mortalities of (pre)mature calves following a gestation length of more than 260 d, which were stillborn or died within 48 h after birth. When a farmer reports an APM case, the sanitary veterinarian must collect a blood sample from the corresponding dam. This blood sample is sent to one of the accredited laboratories along with the associated fetus/calf or placenta (or both) for Brucella-specific analyses. The Budgetary Fund for the Health and Quality of Animals and Animal Products funds the sampling by the veterinarian, and the Federal Agency for the Safety of the Food Chain (FASFC) funds the laboratory *Brucella*-specific analyses.

At the end of 2009, the APM monitoring program was expanded to encompass a broader spectrum of analyses (Table 1). This extension allowed for the monitoring of the most relevant infectious causes of APM in the country (Van Loo et al., 2021). Simultaneously, to streamline the process and increase efficiency, daily sample collection (including maternal blood, placenta, and fetus or calf samples) at the farm level was organized by the diagnostic laboratories. As an incentive, the FASFC fully funds both the on-farm sample pickup and the newly introduced laboratory analyses, in addition to the *Brucella*-specific analyses that were already funded. To stimulate the reporting of bovine APM, a communication campaign to emphasize the importance of APM monitoring was initiated at the end of 2009. This campaign involved distributing information leaflets that highlighted the importance of reporting and submitting cases of APM. Moreover, the renewed APM monitoring program and its related regulations were communicated to cattle farmers, farmer associations, and bovine veterinary practitioners through leaflets, newsletters, and articles in agricultural and veterinary media. Additionally, 2 veterinarians were hired and

stationed at accredited diagnostic regional laboratories. These veterinarians visited cattle herds facing APM issues, particularly those with an annual abortion rate exceeding 5%, or those experiencing a cluster of APM cases within a short time period. During dedicated meetings organized by the involved laboratories, they also educated farmers and their veterinarians on the topic. Between 2010 and 2021, the Belgian APM monitoring program underwent several revisions, resulting in the removal of some analyses (Table 1), mainly because of budget reallocations. However, other analyses, such as a PCR test for Schmallenberg virus (**SBv**) for cases with arthrogryposis-hydranencephaly syndrome, were included.

It is worth investigating whether the new APM monitoring program, its revisions, or other events (such as the outbreak of an emerging abortifacient agent, or the introduction of an eradication program for a specific abortifacient agent) may have affected the number of submitted APM cases. Therefore, a longitudinal observational retrospective cohort study was conducted, utilizing 2 datasets. The first dataset (dataset 1) covered the time period between January 2006 and December 2021, whereas the second dataset (dataset 2) covered the time period between January 2009 and December 2021. Both datasets included the total number of bovine APM submissions, and the total number of calf births in Flanders. The number of APM submissions was extracted from the regional laboratory information management system, and the number of births was available from the national identification and registration system (SANITEL). Notably, cases of APM were not registered as births in SANITEL.

Because no human or animal subjects were used, this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

## Dataset 1 (Type-Year-Season)

For this dataset, the total number of bovine APM submissions and the total number of calf births per month in Flanders were available per production type (dairy, beef, and double purpose) for the time period between January 2006 and December 2021. The production type for each birth and APM case was extracted from the national identification and registration system. The month of submission of APM cases was used to group the APM cases per meteorological season. Winter cases were submitted from December to February, spring cases from March to May, summer cases from June to August, and fall cases from September to November. Data from double-purpose cattle (809,710 births and 2,142 APM cases) were excluded because it was not clear under which type of management these animals were kept.

## Dataset 2 (Herd)

For this dataset, the number of APM submissions and the number of births per year for each active cattle herd in Flanders were collected for the time period between January 2009 and December 2021. A cattle herd was defined as active when at least 1 birth or APM event was registered each consecutive year from 2009 until 2021. A total of 13,664 active herds were available for this time period. Herds with less than 25 reproductive females (cows that gave birth or experienced an APM event) each consecutive year were considered as nonprofessional, and were excluded from the dataset, resulting in a final study population of 4,164 herds. For each year, herds were grouped in quartiles based on herd size (i.e., the number of reproductive females). Herds smaller than the first quartile were classified as small. Medium-sized herds had a total number of bovine females in reproductive age that fell in the interquartile range, whereas large herds were larger than the third quartile. Depending on the years, the first quartile was found between 44 and 53, and the third quartile ranged between 80 and 120 bovine females in reproductive age.

Starting from 2015, the Belgian government implemented the national bovine viral diarrhea virus  $(\mathbf{BVDv})$  eradication program. As part of this program, every newborn calf and aborted fetus underwent mandatory sampling using ear notches to detect BVDv antigen through ELISA or PCR tests. In dataset 2, information on the number of submitted APM cases that tested positive for BVDv and the annual count of immunotolerant persistently infected (**IPI**) calves born at the herd level were available for each herd since 2015.

#### **APM Proportion**

To evaluate the number of APM submissions, an APM proportion  $(\mathbf{APM}_{\mathbf{PR}})$  was calculated by dividing the number of APM submissions by the total number of reproductive bovine females (formula [1]). The total number of reproductive bovine females is the sum of the number of APM submissions and the number of registered births.

 $\frac{\text{number of APM submissions}}{(\text{number of births} + \text{number of APM submissions})} \times 100 =$ 

APM proportion (%)

[1]

					Time	Time period <sup>1</sup>		
${\rm Pathogen}^2$	$\mathrm{Test}^3$	Sample	1978 to 10/2009	10/2009 to 10/2011	10/2011 to $01/2012$	$\begin{array}{c} 01/2012 \ { m to} \\ 03/2012 \end{array}$	03/2012 to $04/2014$	04/2014 to $2021$
Brucella abortus	ELISA Ab SAT Ab ZN staining	Maternal serum Maternal serum Fetal abomasal content or	>>>	>>>	>>;	>>>	>	>
	Culture	placenta Fetal abomasal content or	> >	> >	> >	> >	>	>
BVDv	ELISA Ab Elisa Ac	placenta Maternal serum Eat al calcon		>`			~	
BHV-1	ELISA Ab	Maternal serum		>>`	> `	> `	>	>
BTv SBv	PCR PCR	Fetal spleen Fetal spleen		>	>	>>		$\searrow$
Aerobic bacteria	Culture	Fetal abomasal content and		>	>	~>	~>	~>
	Histology	lungs Fetal lungs		>	$\searrow$	>		
Coxiella burnetii	ELISA Ab PCR.	Maternal serum Fetal abomasal content		~~~	. \*	. ``	/	
<i>Leptospira interrogans</i> serovar Hardio	ELISA Ab	Maternal serum		~>	>	>	>	
Listeria spp.	Culture	Fetal abomasal content and		>	>	>	>	>
Yeast or mold	Culture	lungs Fetal abomasal content		>	>	>	>	$\searrow$
Neospora caninum	ELISA Ab Histology	Maternal serum Fetal heart and brain				>>		>
<sup>1</sup> Check marks indicate the	time period in w	$\frac{1}{2}$ Check marks indicate the time period in which each analysis was performed. The extensive abortion monitoring program was introduced after the time period of 1978 to 10/2009	l. The extensive	abortion monitor	ing program was	introduced after th	le time period of 1	.978 to $10/2009$ .

Table 1. Background of the Belgian abortion monitoring program between the years 1978 and 2021, showing the different pathogens

<sup>2</sup>BVDv = bovine virus diarrhea virus; BHV-1 = bovine herpes virus type 1; BTv = bluetongue virus; SBv = Schmallenberg virus.  $^{3}SAT$  = serum agglutination test; Ab = antibody; Ag = antigen; ZN = Ziehl Neelsen.

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## Statistical Analysis

All statistical analyses were performed using R Studio (version 3.6.1; R Core Team, Vienna, Austria). Binomial generalized linear mixed models were constructed to identify variables that are associated with  $APM_{PR}$ . This was performed within each dataset separately (datasets 1 and 2). In both cases, the dependent variable was  $APM_{PR}$ , and the independent variables were different depending on the dataset. Model fit was evaluated using  $R^2$ .

For dataset 1, the variables were composed of *produc*tion type, year, and season (type\_year\_season model).

For dataset 2, three separate models were constructed each within a specific time period. This allowed the inclusion of an increasing number of variables. Herd ID was defined as a random effect for each of these models. The time periods used in each of the 3 models were the following:

- (1) 2009–2021: With the variables herd size and year, and their interaction (size\_year model).
- (2) 2010–2021: Next to herd size and year variables, APM submissions in the previous year (yes/no) was added in this model (size\_year\_previousyear model). The year 2009 was excluded from this model because no data were available for the previous year (2008).
- (3) 2015–2021: For this model (size\_year\_previousyear\_BVD model), the variable presence of BVDv was added. Presence was established in a yearly window (-1, 0, +1) effect. The BVDv presence was defined as positive when one or more APM events tested positive for BVDv, or when one or more neonatal calves were identified as IPI. For this period, data of BVDv presence at herd level were only available from 2015.

In all models, only significant (P < 0.05) variables were retained. For those variables, least squares means (**LSM**) were calculated and pairwise comparisons were computed using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995). To avoid collinearity, the variation inflation factor (**VIF**) was evaluated for the different variables within each dataset, and the threshold was put at 2.5 (Johnston et al., 2018).

## RESULTS

#### Dataset 1

Between January 2006 and December 2021, a total of 63,221 APM cases were submitted. In 55.7%(35,188/63,221) of the cases, the production type was

beef (>90% Belgian Blue), whereas the type was dairy (>90% Holstein) in 41% (25,891/63,221) and double purpose in 3.4% (2,142/63,221) of the cases. In this time period, 7,699,289 calf births were registered, of which 40.4% were beef, 49.1% dairy, and 10.5% double purpose. The mean  $APM_{PR}$  from 2006 until 2021 was 0.81% ( $\pm 0.26\%$ ). This was 0.68% ( $\pm 0.19\%$ ) for dairy, 1.12% (±0.40%) for beef, and 0.26% (±0.11%) for double-purpose cattle. Before the introduction of the extended analytical panel and the free on-farm sample collection (2006 until 2009), the mean number of submitted APM cases was 2,076 per year (SD =  $\pm 77$ ). The total APM<sub>PR</sub> for this period was 0.44%. This was 0.43% (3,176 submissions vs. 727,588 births) in dairy, 0.47% (3,928 submissions vs. 829,704 births) in beef, and 0.23% (542 submissions vs. 230,460births) in double-purpose cattle. After the introduction of the extended analytical panel and the free onfarm sample collection (2010 until 2021), the mean number of submitted APM cases was 4,805 per year  $(SD = \pm 610)$ . The total APM<sub>PR</sub> for this period was 0.94%, whereas this was 0.79% (17,930 submissions vs. 2,239,318 births) in dairy, 1.37% (24,608 submissions vs. 1,776,009 births) in beef, and 0.33% (1,365submissions vs. 416,385 births) in dual-purpose cattle. Descriptive results of  $APM_{PR}$  per month and per year are depicted in Figures 1 and 2 for both dairy and beef cattle.

No collinearity was detected within this dataset using the VIF. Results from the statistical analyses through the type\_year\_season model are shown in Table 2. R-squared for the type\_year\_season model was 0.65. Our analysis revealed that between 2006 and 2021, the LSM of APM<sub>PR</sub> was higher (P < 0.001) in beef  $(1.05 \pm$ 0.06%) compared with dairy  $(0.61 \pm 0.04\%)$  cattle. The LSM of APM<sub>PR</sub> was the highest (P < 0.001) in winter  $(1.11 \pm 0.08\%)$ , followed by fall  $(0.78 \pm 0.07\%)$ , spring  $(0.71 \pm 0.06\%)$ , and summer  $(0.67 \pm 0.06\%)$ . The LSM of  $APM_{PR}$  of the years between 2006 and 2009 ranged from  $0.41 \pm 0.096\%$  (2006) to  $0.46 \pm 0.102\%$  (2008), whereas this ranged from  $0.84 \pm 0.14\%$  (2020) to 1.23  $\pm 0.17\%$  (2012) between 2010 and 2021. Results of pairwise testing of the LSM of  $APM_{PR}$  per year are depicted in Figure 3, which shows that the LSM of  $APM_{PR}$  was higher in 2012 (1.23  $\pm$  0.17%) and 2015  $(1.20 \pm 0.16\%)$  (P < 0.05) compared with all of the other years.

# Dataset 2

Fourteen percent (589/4,164) of the selected active cattle herds did not submit any APM case for analysis during the analyzed time period (2009 until 2021). Overall, the mean number of APM submissions was Van Loo et al.: ENHANCING BOVINE ABORTION SURVEILLANCE

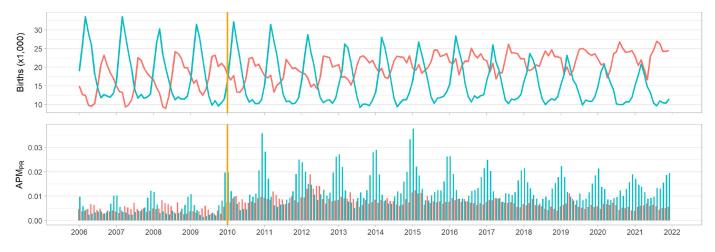


Figure 1. Monthly number of births, and proportion of abortion, stillbirth, and perinatal mortality  $(APM_{PR})$  in Flanders between 2006 and 2021 in beef (blue) and dairy (red) cattle. The extensive APM monitoring program was introduced since 2010 (orange line).

7.7 cases per herd, ranging from 0 to 94, whereas the median number was 5. Of the selected active herds, 33.9% submitted at least 1 APM case per year between 2010 and 2021, ranging from 25.1% (1,047/4,164) in 2010 to 41.2% (1,715/4,164) in 2015, whereas this was only 9.7% in 2009 (405/4,164).

No collinearity was detected within this dataset using the VIF. Results from the statistical analyses through the size\_year model are shown in Figure 4. R-squared for the size\_year model was 0.29. In general,  $APM_{PR}$ was higher (P < 0.001) in small-sized compared with medium and large herds. Differences in  $APM_{PR}$  between small, medium, and large herds were in general more significant (P < 0.05) after the introduction of the extensive analytical panel and the free on-farm sample pickup in 2010. Results of pairwise testing of  $APM_{PR}$ per year, stratified for herd size, are shown in Figure 5. R-squared for the size\_year\_previous year model was 0.25. This model revealed that herds with one or more APM submissions in the previous year had a higher (P < 0.001) APM<sub>PR</sub> in the subsequent year. The size\_ year\_previous year\_BVD model displayed that herds in which at least one APM or a (live) neonatal calf tested positive for BVDv during the defined yearly window had a higher (P < 0.01) APM<sub>PR</sub> compared with herds without a BVDv-positive fetus or calf. R-squared for the size\_year\_previous year\_BVD model was 0.28.

## DISCUSSION

To the best of our knowledge, the present study documents the largest dataset on bovine APM dynamics so far, covering an extensive period of 16 years of surveillance (2006 until 2021).

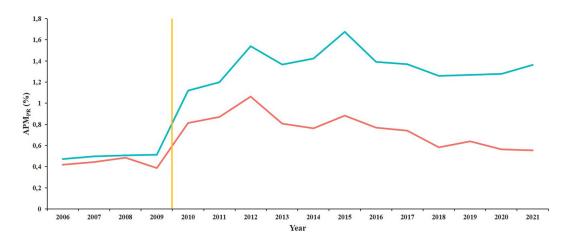


Figure 2. Descriptive statistics showing bovine abortion, stillbirth, and perinatal mortality proportion ( $APM_{PR}$ ) per year (2006–2021) in beef (blue) and dairy (red) cattle in Flanders. The extensive APM monitoring program was introduced since 2010 (orange line).

**Table 2.** Least squares means  $\pm$  SEM of abortion/perinatal mortality proportion (APM<sub>PR</sub>) for each year (2006–2021), season (winter, spring, summer, and fall), and production type (dairy and beef)

Variable	$egin{array}{c} { m APM}_{ m PR} \ ({ m LSM}~\pm~{ m SEM}) \end{array}$
Year	
2006	$0.41 \pm 0.096$
2007	$0.44 \pm 0.100$
2008	$0.46 \pm 0.102$
2009	$0.43 \pm 0.098$
2010	$0.92\pm0.143$
2011	$0.98 \pm 0.149$
2012	$1.23\pm0.167$
2013	$1.03 \pm 0.154$
2014	$1.03\pm0.151$
2015	$1.20 \pm 0.160$
2016	$1.02 \pm 0.146$
2017	$0.99 \pm 0.147$
2018	$0.85 \pm 0.137$
2019	$0.89 \pm 0.141$
2020	$0.84 \pm 0.138$
2021	$0.86 \pm 0.140$
Season	
Winter	$1.11 \pm 0.079$
Spring	$0.71 \pm 0.058$
Summer	$0.67 \pm 0.064$
Fall	$0.78 \pm 0.070$
Production type	
Dairy	$0.61 \pm 0.040$
Beef	$1.05 \pm 0.059$

Our main finding revealed a significant increase in the  $APM_{PR}$  across all production types following the introduction of a more extensive APM monitoring program and an on-farm sample pickup service, both fully funded and promoted by the government. Notably, the submission of APM cases nearly doubled in dairy and almost tripled in beef cattle. Multiple factors could account for this increase in submissions. In a previous study, identifying the cause of an abortion was recognized as the main motivator for farmers to submit samples for APM investigation, rather than just the legal requirement (Clothier et al., 2020). However, in our study, it might be possible that also the availability of on-farm sample collection and the access to accredited and free diagnostic services since the end of 2009 may have been one of the primary motivators to submit a case of bovine APM for analysis. It is worth mentioning that this convenient on-farm sample collection feature was not present in the study conducted by Clothier et al. (2020), where farmers must take the time to bring the fetus to the laboratory, which might be located at a considerable distance. Farmers mentioned laboratory accessibility and the time cost involved in submitting APM samples as a great barrier (Clothier et al., 2020). In the present study, the introduction of daily on-farm sample collection by the diagnostic laboratory (Animal Health Services Flanders) was entirely governmentfunded and concurrent with the implementation of the

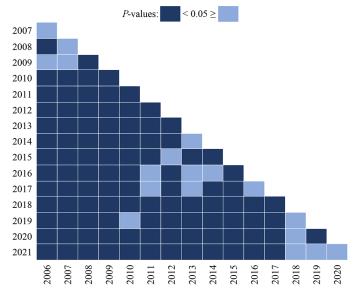


Figure 3. Heatmap showing the results of pairwise testing of abortion and perinatal mortality proportion per year (2006–2021).

extensive analytical panel. Before this initiative, the free on-farm collection of APM cases was not organized by the government. Moreover, broadening the analytical panel of the presented abortion monitoring program might have significantly contributed to the increased  $APM_{PR}$  since 2010. This extension resulted in an increased diagnostic rate of up to almost 40% (Van Loo et al., 2021), which likely encouraged farmers to submit more cases of APM for analysis. Before the implementation of the extensive analytical panel, APM cases were only analyzed for brucellosis, despite the country already being officially declared free of the disease since 2003. This limited focus might have resulted in low farmer interest in participating in the previous abortion monitoring program. Farmers are more likely to prioritize biosecurity measures during an outbreak situation for diseases they are aware of, whereas diseases absent in the country are often considered to pose minimal risk (Ekboir, 1999; Bronner et al., 2014). Furthermore, the intensive communication campaign launched during the early stages of the extensive APM monitoring program may have also contributed to the increased  $APM_{PR}$ . Previous evidence suggests that educating farmers, as well as their veterinarians, may increase the likelihood to report signs of disease (Garner et al., 2016). Based on this, it may be concluded that an accessible, comprehensive, government-funded and -promoted APM surveillance program effectively encourages farmers and their veterinarians to report and submit cases of bovine APM.

In the present study, a higher  $APM_{PR}$  could be observed for the years 2012 and 2015. The higher  $APM_{PR}$ 

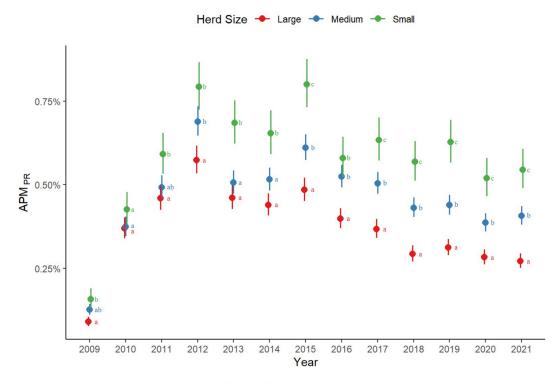


Figure 4. Abortion and perinatal mortality proportion  $(APM_{PR})$  for each year between 2009 and 2021, stratified for herd size (small, medium, large). Herd size was based on the number of reproductive females and categorized based on quartiles (small = first quartile; medium-sized = 2 interquartiles; large = fourth quartile). Different letters (a-c) within the same year indicate a significant difference (P < 0.05).

in 2012 may be explained by the SBv outbreak in Belgium during fall 2011 (Méroc et al., 2013b; Van Loo et al., 2013). Following the implementation of an SBv PCR test in the APM monitoring program and the detection of the first SBv-positive bovine neonate in January 2012, more (malformed) cases of APM were submitted for analysis between January and August 2012 (Van Loo et al., 2013). Beyond August 2012, the number of APM cases with SBv-associated lesions decreased, which might be explained by the findings of Méroc et al. (2013a), who concluded that after the first SBv outbreak in 2011 and 2012, almost every cow in Belgium has been in contact with the virus. The between-herd seroprevalence in 2012 in Belgian cattle was estimated at 99.76%, and the within-herd seroprevalence at 86.3%. Additionally, a long persistence of immunity against the virus after seroconversion of at least 1 year was demonstrated (Méroc et al., 2015).

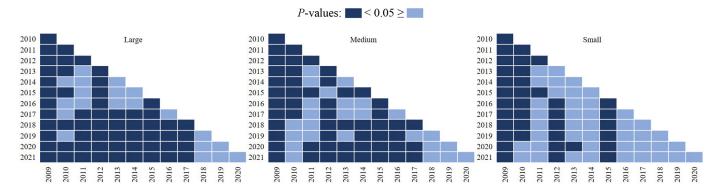


Figure 5. Heatmaps showing the results of pairwise testing of abortion and perinatal mortality proportion (APM<sub>PR</sub>) per year (2009–2021) on large, medium, and small cattle herds. Per year, herd size was based on the number of reproductive females and categorized based on quartiles (small = first quartile; medium = 2 interquartiles; large = fourth quartile).

Consequently, the vast majority of the Belgian cattle population should have developed postinfection protective immunity against the virus. As a result, the number of APM cases associated with SBv decreased after the first outbreak of the virus, which might explain the decrease of APM submissions beyond the peak of 2012. This observation reveals that APM monitoring may be a valuable tool to detect outbreaks of emerging causes of APM, although vigilance of farmers and their veterinarians is essential in initial reporting and submitting of cases to the laboratories. Additionally, it should be noted that agricultural media coverage regarding the clinical consequences of SBv infection may have motivated farmers to report suspected APM cases with potential SBv infection. The second peak in  $APM_{PR}$  in 2015 may be related to the introduction of the mandatory BVDv eradication program in Belgium in the same year. This BVDv eradication program was based on compulsory BVDv analysis of ear notch samples from neonatal calves, but also from cases of APM. Because APM is a potential outcome of an intrauterine BVDv infection (Kelling, 2007; Mee, 2013), reporting and submission for analysis of APM cases were stimulated during the communication campaign of the BVDv eradication program, leading to a higher  $APM_{PR}$  at the beginning of this program. For the years beyond the beginning of the mandatory BVDv eradication, it could be observed that herds where BVDv was detected in an APM case or in a (live) neonatal calf had a higher  $APM_{PR}$ . This may be explained by the fact that BVDv is a well-known abortifacient agent in cattle. As a result, the presence of the virus in a cattle herd may lead to a higher  $APM_{PR}$ . Another explanation for this finding may be that having previous personal experience with the disease may increase the likelihood that involved farmers correctly identify clinical signs (e.g., APM), leading to a higher submission of APM cases (Guinat et al., 2016; van Andel et al., 2020; Gates et al., 2021).

Interestingly, we observed a decrease in  $\text{APM}_{\text{PR}}$  after the year 2015, especially in large-sized farms. This decrease may be explained by the fact that it might be difficult to sustain long-term engagement with disease reporting in enhanced passive surveillance programs like the presented abortion monitoring program, even when incentives are provided (Gates et al., 2021). Also, the removal of some analyses from the analytical panel since October 2011 may have had a negative effect on the motivation of farmers to submit an APM case, resulting in a decreased number of submissions, although this could not be fully substantiated from the results of the present study.

In the present study, we observed that the  $APM_{PR}$  was higher in beef compared with dairy cattle, which

corresponds with the findings of Sarrazin et al. (2014), who reported that Belgian beef farmers were more inclined to submit each case of abortion (88%), compared with dairy farmers (42%). Clothier et al. (2020) also identified a higher motivation among beef farmers to submit APM cases for analysis compared with dairy farmers. In contrast, other studies observed that dairy farmers were more likely than beef farmers to contact a veterinarian to report (re)-emerging diseases (Gilbert et al., 2014) and cases of APM (Bronner et al., 2013). This discrepancy could not be clearly explained, but the higher submission rate of APM cases in beef cattle in the present study may be attributed to the high proportion of Belgian Blue cattle in the Belgian beef cattle population. Belgian Blue calves hold a higher economic value compared with most other beef cattle breeds, and especially when compared with dairy breeds, with the price of Belgian Blue beef calves being about a 9-fold higher than that of dairy calves. This may make Belgian Blue beef cattle breeders more inclined to submit APM cases for further analysis to find out the underlying cause of fetal or calf mortality. Moreover, in beef cattle, the calf is the primary source of income, leading to much more focus on the birth of a healthy calf in this production type. Both male and female calves are important in beef cattle, whereas in the dairy industry, male calves are often considered by-products destined for the veal calf or dairy beef industry. The latter may lead dairy farmers to be less interested in the cause of an APM in a male calf. Unfortunately, we did not have information on the fetal sex of the included cases to further investigate this aspect. Furthermore, the management practices in beef cattle vary from those in the dairy industry in several ways, including housing, vaccination rate, and nutrition. These variations may lead to a difference in prevalence of certain abortifacient pathogens in both production types (Van Loo et al., 2021), which may also explain the differences in  $APM_{PR}$  observed between beef and dairy cattle in our study.

A seasonal distribution in  $\text{APM}_{\text{PR}}$  was observed, with a higher number of cases submitted during fall and winter compared with spring and summer. This may be attributed to the breeding season on pasture typically applied in many Belgian Blue beef herds between April and October. During this period on pasture, the detection and reporting of abortions may decrease, as mentioned by Bronner et al. (2014). Additionally, due to the typical breeding season, most of the Belgian Blue cows are nonpregnant or in the first trimester of gestation in spring and summer. Previous studies (Forar et al., 1996; Norman et al., 2012; Mee, 2020) reported that detecting fetal loss is less likely in early pregnancy, which could be another reason for the observed seasonal submission pattern of beef cases.

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To calculate the  $APM_{PR}$ , we assumed that all detectable APM cases were submitted for analysis. However, one of the limitations of the present study is that we believe that the submitted numbers do not fully reflect the actual APM numbers. Previous research has suggested that the real number of abortions may be approximately 2.2 to 5 times higher than the submitted number (Forar et al., 1995; Kinsel, 1999). This estimation is based on the consideration that when only observed abortions are taken into account, normal abortion rates beyond 120 d of gestation appear to be around 2% to 5% (Kinsel, 1999; Hovingh, 2009; Mee, 2020). Additionally, perinatal mortality rates after 265 d of gestation have been reported to vary between 2.4%and 9.7% (Cuttance and Laven, 2019). In the present study,  $APM_{PR}$  was analyzed in commercial herds with at least 25 reproductive females per year, which means that at least 1 APM event every 2 years on each commercial herd should have been happened, assuming a minimal APM rate of 2%. However, 14% of the included herds never submitted any APM case over a period of 13 years, suggesting that even under mandatory conditions, where collection and extensive analysis of the samples were completely funded by the government, many APM cases remain unreported. Even with mandatory reporting, farmers and their veterinarians often fail to report APM cases, guided by self-interest and other reasons such as health aspects, financial loss, practical obstructions, peer influence, and fear of consequences such as farm isolation in case of reporting of a suspected case of brucellosis or another notifiable pathogen (Elbers et al., 2010; Bronner et al., 2014). Moreover, depending on risk aversion, some farmers need multiple cases of APM before deciding to report. Furthermore, especially in large herds, sporadic abortion is considered to be a normal event, and farmers are not prompted to report each case (Bronner et al., 2014). The latter is confirmed by the present study, where a lower  $APM_{PR}$  was observed in larger compared with smaller herds. However, this is in contrast with a previous study from the United Kingdom, where it was found that farmers from larger dairy herds were willing to pay more for APM analysis, potentially due to the larger impact on the herd, or the overall more intense herd health management taking place in larger dairy cattle farms (Clothier and Anderson, 2016; Clothier et al., 2020). Based on this, Clothier et al. (2020) assumed that the motivation to investigate cases of APM will remain or grow, as there is a trend toward larger herds. Although there is no clear explanation for our contradictory finding, there may be a correlation with the growing issue of staff retention and turnover in large-scale, labor-intensive dairy farms (Tipples et al., 2010, 2012). Because of this, it could be possible that the employed staff on larger commercial farms is less experienced (or motivated) with recognizing abnormal behaviors and clinical signs of disease (e.g., abortion) compared with the owner of a smaller family-owned and operated farm (Gates et al., 2021). All these factors may cause underreporting of APM cases, which makes it challenging to estimate the real prevalence of APM.

To ensure freedom of brucellosis with a 99% confidence level, Welby et al. (2009) calculated that a minimum of 8,000 submitted APM cases per year would be required in Belgium, with 4,000 each in Flanders and Wallonia, in combination with the other components within the national brucellosis surveillance system (e.g., serological analyses). However, in the present study, before the introduction of an extensive analytical panel and the on-farm sample pickup, the mean number of submitted APM cases per year in Flanders was only 2,076. Broadening the analytical panel and free on-farm sample collection resulted in an increase of the mean number of submitted cases per year up to 4,576, which seems to be sufficient to guarantee the brucellosis-free status in Flanders.

#### **CONCLUSIONS**

This study offers a general overview and valuable insights from the bovine APM screening approach in Flanders, Belgium, with potential implications for global surveillance programs. However, region-specific factors, such as the presence of Belgian Blue cattle, and unique program features, such as a free on-farm sample pickup service and the fully funded extended analytical panel, may limit direct application elsewhere. Despite these limitations, the study concludes that an accessible, comprehensive, and cost-effective APM surveillance program with a reasonable diagnostic rate encourages reporting of APM cases. However, even with mandatory reporting, underreporting challenges persist, urging the identification of drivers to improve submission rates and ensure the effectiveness of APM monitoring programs, particularly for infectious and zoonotic causes.

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