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Emergence of fatal *Mannheimia haemolytica* infections in cattle in the Netherlands

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ABSTRACT

In the Dutch national surveillance system, outbreaks of fatal infections by *Mannheimia haemolytica* (*M. haemolytica*) in dairy cows and veal calves have become apparent in recent years. These observations prompted an in-depth analysis of available pathology data over the period 2004–2018 to investigate changes in the occurrence and/or expression of *M. haemolytica*-associated cattle disease. With multilevel logistic regression models, time trends were identified and corrected for farm, season, pathologist and region.

Deaths associated with *M. haemolytica* infection increased over time with dairy cows and veal calves diagnosed with fatal *M. haemolytica* infections 1.5 and 1.4 times more frequently every following 3-year period between 2004 and 2018, respectively. *M. haemolytica*-associated disease showed two distinct disease presentations: acute pleuropneumonia in dairy cows and polyserositis in veal calves. The prevalence of both disease presentations with *M. haemolytica* confirmed increased in each 3-year time period between 2004 and 2018, with an odds ratio (OR) of 1.5 for acute pleuropneumonia in dairy cows and an OR of 1.7 for polyserositis in veal calves. No change was found for *M. haemolytica*-associated disease in dairy calves. Although *M. haemolytica* is considered an opportunist bovine pathogen, and the presence of primary pathogens such as BHV-1, BVDV and *Mycoplasma* species was not completely ruled out in our study, substantial evidence is provided to indicate infections with *M. haemolytica* were the most likely cause of death. *M. haemolytica*-associated diseases occurred more often in October–June than July–September, and were detected more often in necropsied animals from the North, South and East Netherlands than the West Netherlands.

Introduction

Mannheimia haemolytica (*M. haemolytica*), previously known as *Pasteurella haemolytica*, is a commensal bacterium of the upper airways of ruminants (Timsit et al., 2016). However, this bacterium may also cause acute and often fatal infections in bovines and small ruminants (Zecchinon et al., 2005). Since 2002, Royal GD has operated a national surveillance system for the health of farm animals in the Netherlands (Santman-Berends et al., 2016; Linde van der et al., 2018), of which necropsy of dead farm animals is an important component. The GD Laboratory for Pathology and Histology is the only site in the Netherlands that has carried out numerous necropsies on cattle each year and findings are registered in a database. This data includes reports on clinico-pathological and epidemiological features of fatal infections by *M. haemolytica* in cattle in the Netherlands.

In recent years, veterinarians at GD were alarmed by farm-associated outbreaks of acute pleuropneumonia in dairy cows. These outbreaks sometimes resulted in fatalities in dozens of highly productive dairy cows within a short time period. Initially, veterinarians considered these outbreaks may be caused by bovine alpha herpes virus-1 (BHV-1) infections, but in all outbreaks conjunctival and/or nasal swabs of diseased cows tested negative for this agent by PCR ([1] ; Royal GD, Monitoring Registration Database, unpublished results). From necropsy samples, *M.*

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[1] See: Royal GD, Monitoring Cattle Health (In Dutch). https://edepot.wur.nl/168562 (accessed 12 November, 2020)

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haemolytica was isolated in heavy growth from affected lung tissue. Additionally, pathologists observed an increased number of veal calves suffering from severe polyserositis with *M. haemolytica* isolated from pericardium, pleura and/or the peritoneum showing acute fibrinous inflammation (Royal GD, Pathology Database, unpublished results). These observations lead to an in-depth analysis of the GD pathology data to further investigate possible changes in occurrence and/or clinical manifestation of *M. haemolytica*-associated disease in cattle in the Netherlands. In this investigation, the aim was to identify trends in numbers and prevalence of *M. haemolytica* infections in necropsied cattle over the period 2004–2018.

Materials and methods

Type of data

Necropsy reports (n = 31,483) of cattle over the period 2004–2018 were available for analysis at GD. This data included information of submission-date, case identification number, unique farm number, farm-type (dairy cow/calf, suckling cow/calf, veal or other), cattle-type, age, pathological findings and etiological diagnosis and pathologist.

Necropsy examinations

For necropsy, dead animals were reported by farmers or their attending veterinarians, and transported to GD for necropsy. Necropsies were undertaken by board-certified veterinary pathologists (with either national RNVA or international EBVS registration) under ISO-17025 accreditation. For reasons of efficiency and diagnostic cost-effectiveness each necropsy was carried out in a standardized order (termed primary, secondary and tertiary screens). A more detailed description of this process is provided in Supplementary methods.

Bacteriology

Samples for bacteriology were taken when findings in the primary screen of the necropsy process suggested bacterial infection, according to standard operating procedures. A more detailed description of the bacteriology is provided in Supplementary methods.

Data validation

Cattle were not always adequately classified on the submission form. Therefore, we defined the age category and cattle-type as follows. Based on the age (in days) provided on the submission form (n = 30,802 cases), cattle originating from dairy- or suckling cow herds were reclassified into two groups, either calf ($\leq$365 days) or cow (>365 days). In 681 cases, age in days was missing. Where the details of these animals contained the words 'calf seven months in gestation', 'abortion', 'calf in uterus' or 'aborted calf', the record was removed. When age in days was missing and the cattle-type was dairy- or suckler cow, the animal was allocated as cow. The remaining 551 animals were classified as cow or calf by the registered cattle-type on the submission form. In addition, veal calves were classified as imported or not by the country of origin derived from the identification number of the animal.

Geographical location of the farm was determined from the unique farm number and four regions of the Netherlands were identified from the following provinces: North (Friesland, Groningen and Drenthe), East (Overijssel, Gelderland and Flevoland), South (Limburg and Noord-Brabant) and West (Noord-Holland, Zuid-Holland, Utrecht and Zeeland). Time of the year was based on quarters, with data extracted from the date of arrival for necropsy and included in the model to correct for possible temporal variations in *M. haemolytica* disease prevalence.

Seventeen veterinary pathologists were employed at GD during the study period. Four pathologists performed <100 bovine necropsies each and were grouped together. The others performed between 469 and 7165 bovine necropsies each. The number of cattle confirmed with *M. haemolytica* infection was plotted against the total number of cattle sent for necropsy per time period to investigate a possible trend over time. Trends were determined for various farm types and age classes separately: dairy calves (<365 days), dairy cows (>365 days), veal calves and other (beef bulls, other cattle-types and unknown).

For each animal diagnosed with *M. haemolytica* infection, a pathological diagnosis (e.g. acute pleuropneumonia, polyserositis) was assigned, allowing analysis of trends for disease presentations and for farm type separately.

Since acute pleuropneumonia and polyserositis may also be caused by other infectious agents, the total number of deaths for each disease presentation associated with *M. haemolytica* was compared against the total number of deaths caused by all infectious agents. By plotting the total number of cases for each disease presentation caused by all possible infectious agents against the total number of cases submitted for necropsy each year, trends over time in the occurrence of *M. haemolytica* infections were examined. This enabled the identification of real increases in *M. haemolytica* infections compared with a more general time trend in the appearance of acute pleuropneumonia or polyserositis.

Statistical analysis

Three multilevel logistic regression models were used to determine trends over time. The outcome variable in all models was binomially distributed. In the first model the outcome variable was whether or not the pathological findings of the animal were associated with a *M. haemolytica* infection. The second model was applied on two different subsets of animals in which either all animals had a cause of death related to acute pleuropneumonia or polyserositis. In this case the outcome variable was whether or not the disease presentation in the animal was associated with *M. haemolytica* infection. In the third model the outcome variable was whether or not the pathological findings of the animal were associated with acute pleuropneumonia or polyserositis in general.

In all three models the explanatory categorical variables were period, quarter of the year, region, pathologist and type of cattle. For veal calves, an additional binary variable (imported or not) was added to the model. Two-way interactions were tested between type of cattle and time period.

In the early years, the number of *M. haemolytica* infections was low. Therefore, years were grouped for further analysis. First, year was divided into five equal periods of 3 years as follows: period 1, 2004–2006; period 2, 2007–2009; period 3, 2010–2012; period 4, 2013–2015; and period 5, 2016–2018. Secondly, year was divided in three equal periods of 5 years as follows: period 1, 2004–2008; period 2, 2009–2013; and period 3, 2014–2018. Thirdly, year was divided in two periods as follows: period 1, baseline period (2004–2014); and recent period, (2015–2018). The fit of the models was determined by the log likelihood and Akaike Information Criteria (AIC) and the best fitting model is presented, which is the model with the highest log likelihood and lowest AIC. The explanatory variable time period was added to the model as a categorical variable or as a continuous variable. Time as a continuous variable was preferred but only when a linear association between time period and the number of deaths caused by a *M. haemolytica* infection was present according to qq-plots and when no interaction with time period was significant.

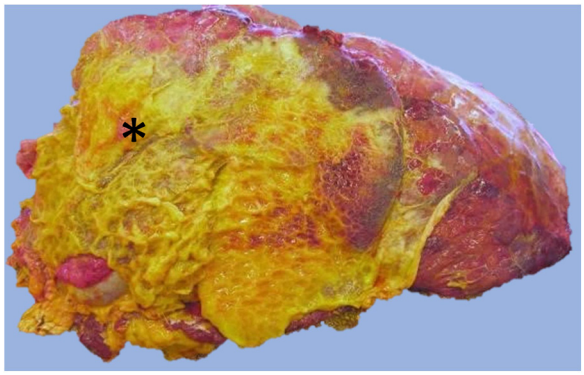

Fig. 1. *M. haemolytica* acute fibrinous pleuropneumonia in a highly productive dairy cow (left lung, lateral aspect). Large pleural deposits of yellowish fibrin are present in the cranial and dorsocranial areas (*). Inflamed pleura is voluminous and has not collapsed after removing the lung from the carcase. The failure to collapse, increased firmness and a dark reddish colour of lung tissue is grossly consistent with pneumonia.

Unique farm number was included as a random effect to account for clustering of animals within farms. Results were expressed as odd ratios (OR) and considered statistically significant at $P \leq 0.05$. All data analyses were performed using statistical packages (SAS 9.3 and StataCorp, 2015, Stata: release 15).

Results

For the case descriptions of acute fibrinous pleuropneumonia caused by *M. haemolytica* in dairy cows and acute polyserositis caused by *M. haemolytica* in veal calves, refer to Supplementary methods (Figs. 1–4).

Descriptive statistics

After data validation, information from 31,319 unique cattle was available (Table 1). Most of the cattle sent for necropsy were dairy calves (40%), dairy cows (28%) and veal calves (27%). Submissions were obtained from all parts of the Netherlands and followed the herd densities in the region (results not shown). *M. haemolytica* infection resulting in death was diagnosed in 2503 cattle (Table 1), originating from 1241 different Dutch farms.

M. haemolytica *infections over time*

The numbers of *M. haemolytica* infections are plotted for each cattle-type against the time period (Fig. 5). The data suggests an increase over time, especially for dairy cows and veal calves. The number of deaths associated with a *M. haemolytica* infection in dairy calves was increasing as well, but decreased again after 2013.

Results of the model with year divided into five equal periods of 3 years gave the best fit and only these results are presented. Results in tabular form are presented in the Supplementary methods. The number of deaths associated with *M. haemolytica* infections was significantly higher in different time periods for different types of cattle. In the period 2016–2018, the odds of diagnosing an adult dairy cow with an *M. haemolytica* infection was 5.2 times higher (95% confidence interval [CI] 3.3–8.2, $P < 0.01$) than in 2004–2006. For these two time periods, the odds of dairy calves and veal calves diagnosed with *M. haemolytica* infection were 1.8 times (95% CI, 1.2–2.7; $P < 0.01$) and 3.2 times higher (95% CI, 2.4–4.3; $P < 0.01$), respectively. No significant differences were found over time for suckler cows and other cattle-types. There was a significant effect of quarter of the year and region. *M. haemolytica* infections were diagnosed significantly more often in the first, fourth and second quarter compared to the third quarter (first quarter OR, 2.1; 95% CI, 1.8–2.4; fourth quarter $P < 0.01$; OR, 1.7; 95% CI, 1.5–1.9; $P < 0.01$; second quarter OR, 1.2; 95% CI, 1.0–1.4; $P = 0.02$) and affected farms were more often located in northern, southern and eastern provinces, compared to the western provinces of the Netherlands (northern provinces OR, 1.7; 95% CI, 1.3–2.1; $P < 0.01$; southern provinces OR, 1.9; 95% CI, 1.5–2.4; $P < 0.01$; eastern provinces OR, 1.8;

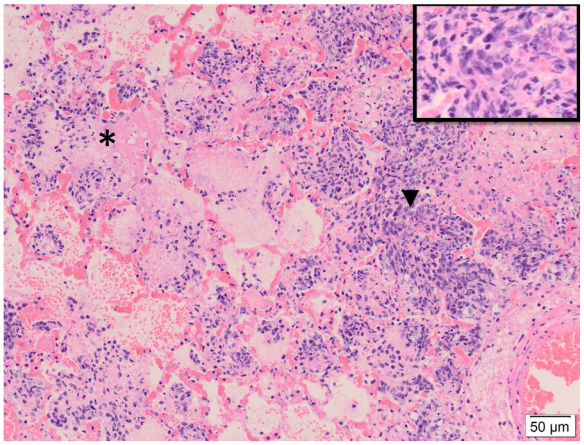

Fig. 3. Histological appearance of inflamed lung tissue in acute *M. haemolytica* fibrinous pleuropneumonia in a dairy cow. H&E stain. Proteinacous, fibrin-containing exudate fills the alveolar lumina (*) and infiltrating neurophils demonstrate a characteristic form of cellular degeneration and lysis ('oat cells'; insert) due to *M. haemolytica* leukotoxin (▼).

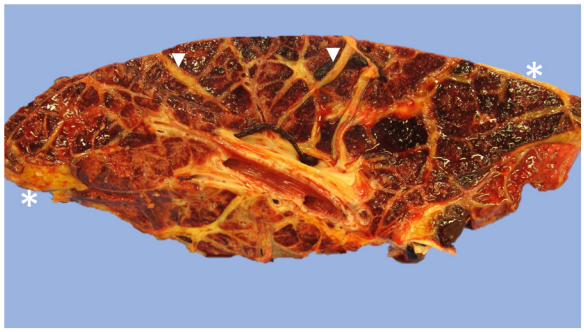

Fig. 2. Cut surface of lung tissue with acute fibrinous pleuropneumonia caused by *M. haemolytica*. Pleural deposit of fibrin (*), and widening of interlobular septae due to fibrinous exudate (▼). The lung parenchyma is voluminous and demonstrates a dark reddish colour.

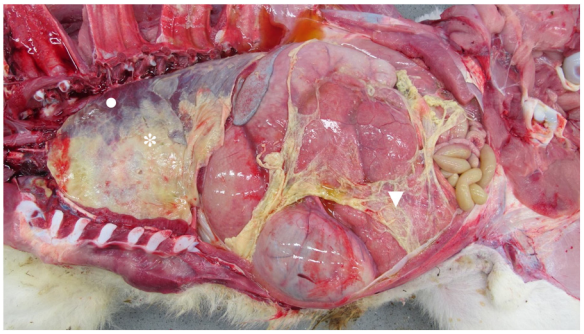

Fig. 4. Acute polyserositis caused by *M. haemolytica* in a veal calf. Acute fibrinous pleuritis (*) and peritonitis (▼). An acute fibrinous pericarditis is often present, but not visible in this view due to large fibrin deposits on the pleura, masking the pericardium. Note the small and dark red appearance of the lung tissue (●), indicating compression atelectasis of the lung tissue due to the fibrinous exudate in the thoracic cavity.

Table 1
Number of carcase submissions and number and percentage of carcases with *M. haemolytica* infection per cattle-type in the period 2004–2018.

Period	Category	Carcase submissions (*n*)	*M. haemolytica* infections (*n*)	*M. haemolytica* infections (%)
2004–2006	Veal calf	1015	87	8.6
	Dairy calf <1 year	2078	50	2.4
	Dairy cow >1 year	1310	26	2.0
	Suckler cow	238	7	2.9
	Other	119	2	1.7
2007–2009	Veal calf	1204	112	9.3
	Dairy calf <1 year	2452	81	3.3
	Dairy cow >1 year	2069	55	2.7
	Suckler cow	217	1	0.5
	Other	159	12	7.6
2010–2012	Veal calf	1271	198	15.6
	Dairy calf <1 year	3055	183	6.0
	Dairy cow >1 year	1665	45	2.7
	Suckler cow	179	8	4.5
	Other	179	6	3.4
2013–2015	Veal calf	2798	595	21.3
	Dairy calf <1 year	2505	129	5.2
	Dairy cow >1 year	1691	115	6.8
	Suckler cow	221	6	2.7
	Other	213	22	10.3
2016–2018	Veal calf	2216	494	22.3
	Dairy calf <1 year	2272	85	3.7
	Dairy cow >1 year	1,921	169	8.8
	Suckler cow	156	4	2.6
	Other	116	11	9.5
Total		31,319	2503	6.6

95% CI, 1.4–2.3; $P < 0.01$). The interclass-correlation of the random farm-effect was 0.20 (95% CI, 0.18–0.24), indicating cattle within the same farm are more likely to be infected with *M. haemolytica* compared to cattle between farms.

M. haemolytica *infections per cattle-type*

Dairy cows and calves

The number and percentage of deaths attributed to *M. haemolytica* infections in necropsied dairy cows >1 year increased significantly over time (range, 0.7–1.8% in 2004–2012; range, 2.9–4.6% in 2013–2018; $P < 0.01$; Fig. 6). In each 3-year period, the odds of a dairy cow having a fatal *M. haemolytica* infection increased on average by 1.5 (95% CI, 1.4–1.7).

In dairy cows >1 year, the most common disease presentation of *M. haemolytica* infection was acute pleuropneumonia. For each time period of 3 years, the odds of diagnosing a dairy cow with acute pleuropneumonia resulting from *M. haemolytica* infections significantly increased (OR, 1.5; 95% CI, 1.2–1.8; $P < 0.01$; Fig. 7). However, the odds of dairy cows with acute pleuropneumonia caused by all infectious agents also increased over time, but with a lower average OR per time period of 1.2 (95% CI, 1.1–1.3; $P < 0.01$; Fig. 8). Most (65%) of the dairy cows with death attributed to *M. haemolytica* were also cultured for *Mycoplasma* spp., with 6.4% testing positive from lung tissue. The number of dairy cows with polyserositis was limited, and no further analysis of those cases was performed.

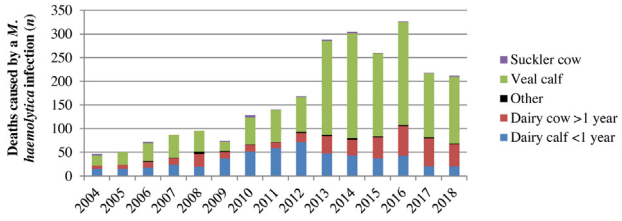

Fig. 5. Number of deaths associated with *M. haemolytica* infections per cattle-type in 2004–2018.

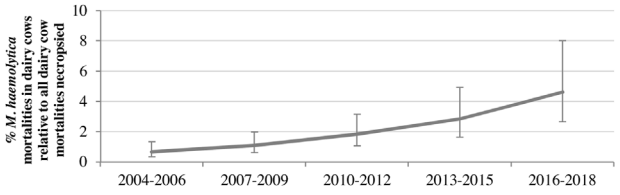

Fig. 6. Predicted % and 95% confidence interval of deaths associated with *M. haemolytica* infections in dairy cows relative to the total number of dairy cows necropsied in 2004–2018.

No significant differences in the number and percentage of deaths associated with *M. haemolytica* infections over time were found in dairy calves ($P > 0.05$; Fig. 9). A slight increase in prevalence was evident between 2010–2012, but this declined between 2013–2018.

Veal calves

The number and percentage of deaths attributed to *M. haemolytica* infections in necropsied veal calves significantly

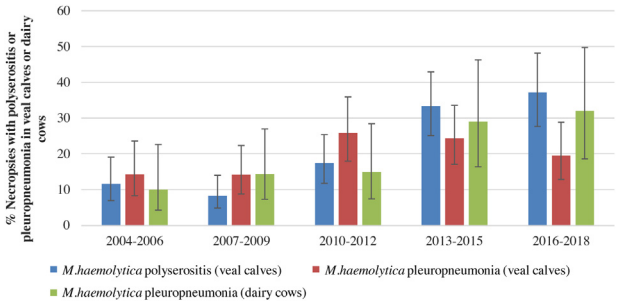

Fig. 7. Predicted % and 95% confidence interval of necropsied dairy cows and veal calves with *M. haemolytica* pleuropneumonia or *M. haemolytica* polyserositis relative to all necropsied dairy cows and veal calves with pleuropneumonia or polyserositis in 2004–2018.

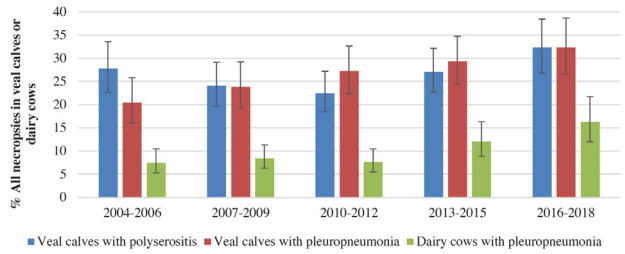

Fig. 8. Predicted % and 95% confidence interval of dairy cows and veal calves diagnosed with pleuropneumonia or polyserositis relative to all dairy cows and veal calves necropsied in 2004–2018.

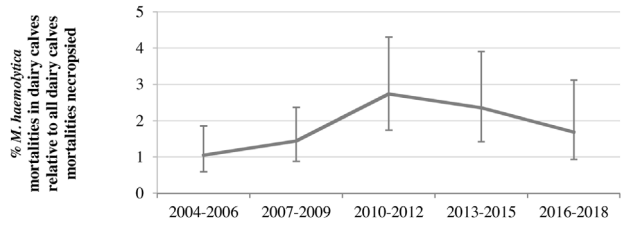

Fig. 9. Predicted % and 95% confidence interval of deaths associated with *M. haemolytica* infections in dairy calves relative to the total number of dairy calves necropsied in 2004–2018.

increased over time and ranged between 7.3–9.8% in 2004–2009 and between 12.3–20.6% in 2010-2018 ($P < 0.01$; Fig. 10). For each 3-year time period, the odds of a veal calf diagnosed with *M. haemolytica* infection increased significantly (OR, 1.4; 95% CI, 1.3–1.5; $P < 0.01$). No significant difference was found between calves born in the Netherlands and imported calves ($P = 0.21$).

From disease presentation data, the percentage of polyserositis associated with *M. haemolytica* infections also increased over time (Fig. 7). For each 3-year time period, the odds of polyserositis cases in veal calves positive for *M. haemolytica* increased significantly (OR, 1.7; 95% CI, 1.5–1.9; $P < 0.01$). In contrast, the odds of veal calves diagnosed with polyserositis caused by all infectious agents in general did not significantly increase over time ($P = 0.06$; Fig. 8).

Apart from a significant increase in 2007–2012 ($P < 0.01$), no increase in the percentage of veal calves with acute pleuropneumonia associated with *M. haemolytica* infections was observed over time (Fig. 7). However, the percentage of veal calves having acute pleuropneumonia caused by all infectious agents increased over time, with the odds of a veal calf having acute pleuropneumonia for each period of 3 years increasing by 1.1 (95% CI, 1.1–1.2; $P < 0.01$; Fig. 8).

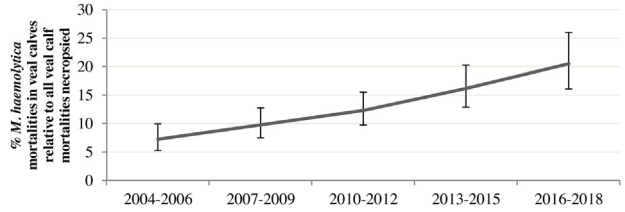

Fig. 10. Predicted % and 95% confidence interval of deaths associated with *M. haemolytica* infections in veal calves relative to the total number of veal calves necropsied in 2004–2018.

Of 71 veal calves with *M. haemolytica* associated polyserositis investigated for a co-infection with *Mycoplasma* spp., 39 (55%) were culture positive (from lung sampling).

Discussion

From the historical pathology data, increases in two distinct disease presentations of *M. haemolytica* infections were recognized. Firstly, *M. haemolytica* was associated with acute pleuropneumonia in highly productive dairy cows. This type of pneumonia was reported by veterinarians and farmers to have a fulminant course, with death often within 24 h after the first clinical signs. Secondly, *M. haemolytica* was associated with fatal polyserositis in veal calves, characterized by acute pleuritis, peritonitis and pericarditis.

Analyses were performed on three main groups of cattle (dairy cows, dairy calves and veal calves) with a significant increase evident in the number and percentage of cases of *M. haemolytica* infections in dairy cows and veal calves. In dairy cows, an increase in the odds of fatal acute pleuropneumonia associated with a *M. haemolytica* infection was found, being 1.5 (95% CI, 1.2–1.8) per 3-year period. Meanwhile, the odds of dairy cows presented to GD with a diagnosis of acute pleuropneumonia irrespective of bacterial aetiology has also increased over time, albeit at a lower rate per 3-year period of 1.1 (95% CI, 1.1–1.3). No significant differences in the number and percentage of cases of *M. haemolytica* infections over time were found in dairy calves.

In veal calves, our results indicate an increased odds of 1.7 (95% CI, 1.5–1.9) per 3-year period of polyserositis associated with *M. haemolytica* infections, while the odds of veal calves presented to GD with a diagnosis of polyserositis irrespective of bacterial aetiology has not increased over time.

The total number of necropsied cattle having a fatal *M. haemolytica* infection increased over time. However, numbers declined in 2017 and 2018, which may be due to a genuine reduction in the number of cases or a growing awareness by veterinarians and farmers. For example, if the initial cases are diagnosed with *M. haemolytica* infection, additional cases with similar clinical signs from the farm will generally not be submitted for necropsy. This will likely result in an underestimation of the number of animals suffering from fatal *M. haemolytica* infection in recent years.

Stress induced by management, such as transport or feed change, physiological stress induced by a high production, climate or unidentified microbial agents could increase *M. haemolytica* replication resulting in inhalation of bacterial-laden droplets into the lungs (Zecchinon et al., 2005; Singh et al., 2011; Taylor et al., 2010). Management that reduces such risk factors could result in a decline in cases associated with *M. haemolytica* infections. This organism appears to affect multiple cows within the same farm, and cattle from the same herd are more likely to be infected compared to cattle from different herds as confirmed by the intraclass correlation of the random herd effect. However, it is not known whether this is caused by common farm factors, or whether it is mainly attributable to horizontal transmission of infection.

The GD receives carcases from farms across the Netherlands with herd health problems. While over the years, absolute numbers of submitted carcases have declined, in line with the decreasing number of cattle farms in the Netherlands, there are no indications of changed submission patterns over time. Therefore, the data are assumed to be representative of Dutch dairy herds that experience health problems resulting in deaths (Cameron et al., 2003).

In May 2012, bacterial identifications at GD were supplemented by matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry. Therefore, the time period

before and after the introduction and validation of MALDI-TOF was added to the models. Both the five 3-year periods and MALDI-TOF effect were significant and the effects could not be separated from each other. The AIC of the model with and without the MALDI-TOF effect did not differ and therefore it was decided to exclude the MALDI-TOF effect from the models. For *M. haemolytica*, both biochemical identification and identification by MALDI-TOF appeared to be relatively clear cut and decisive in the vast majority of cases. In practice, the highest risk of failure to isolate *M. haemolytica* from infected material is overgrowth by fast growing bacteria like *Enterobacteriaceae* or *Pasteurella multocida*, due to the larger colony size of these bacteria. Colony selection precedes colony identification either by biochemical procedures or with use of MALDI-TOF. Therefore, the introduction of the MALDI-TOF is assumed to be of limited influence on the detection of *M. haemolytica.*

Other pathogens can cause severe respiratory signs in dairy cows, including BHV-1 infection. However, the necropsy findings in the primary screen that result from an infection with BHV-1 differ from those of *M. haemolytica*, with BHV-1 causing rhinitis, keratitis, and tracheitis in addition to pneumonia. Since the GD Laboratory for Pathology and Histology uses specifically selected, cost-effective diagnostic test procedures, no further testing of BHV-1 virus was done when no typical signs of BHV-1 were present.

It is difficult to confirm *M. haemolytica* as the primary cause of the reported disease presentations. In the current study design, based upon targeted routine necropsy diagnostic procedures with limited application of diagnostic test for exclusion, investigations into (fulfilment of) Koch's postulates are not feasible. However, primary BHV-1 and bovine viral diarrhoea virus (BVDV or Pestivirus) infections are unlikely to play a major role in the pathogenesis of fatal *M. haemolytica* infections in cattle in the Netherlands. Firstly, in only relatively few animals necropsy findings in the diagnostic pathology screens were suggestive of a BHV-1 or BVDV infection. Secondly, out of 31,319 cattle submitted for necropsy, 109 animals were tested for BHV-1 infection of which 93 tested positive and in the case of BVDV, 302 cattle of this cohort were tested of which 259 were positive. From these BHV-1 and BVDV cases, *M. haemolytica* co-infection was identified in only six and five cases respectively. Thirdly, the prevalence of BHV-1 and BVDV in Dutch farms is declining substantially. Between 2007 and 2011, the herd prevalence of active BVDV infections in dairy herds with an unknown status decreased from 23% to 16% by a voluntary control programme (Van Duijn et al., 2019). Additionally, since 2018, participation in control programmes for both these viruses is mandatory for all Dutch dairy farms, resulting in an increase of BHV-1 and BVDV-free and unsuspected herds. In December 2018, 76% and 77% of the Dutch dairy herds were BVDV and BHV-1 free or unsuspected respectively,[2] compared to 23% for BVDV in 2007 (Van Duijn et al., 2019).

Together, these findings warrant the conclusion that potentially fatal infections by *M. haemolytica* should be included in the differential diagnosis of acute fibrinous pleuropneumonia in dairy cows and in polyserositis in veal calves, and that agents such as BHV-1 or BVDV are unlikely to play an important role in the observed increase of fatal *M. haemolytica* in the Netherlands.

Whether emergence of fatal *M. haemolytica* infections is specific for the Netherlands, or is also happening elsewhere is unclear. Two outbreaks of acute pleuropneumonia associated with a *M. haemolytica* infection, affecting in total 10 dairy cows, were reported in Scotland (SAC C VS Disease Surveillance Report, 2014). In these cases, PCR tests for respiratory viruses proved to be negative. This may indicate that fatal infections with *M. haemolytica* occur and may be emerging in more countries.

Of the number of veal calves that died with a polyserositis associated with *M. haemolytica* infection, only a limited number were investigated for pulmonary co-infection with *Mycoplasma* spp., of which 55% cultured positive. Therefore, it cannot be ruled out that *Mycoplasma* spp. play a role in the pathogenesis of *M. haemolytica* polyserositis, possibly by affecting the mucosal barrier of the respiratory tract. However, it has been shown that *M. haemolytica* strains also have invasive capacities (Cozens et al., 2019).

Antibiotic use in farm animals is a topic of high concern in the Netherlands and legislation on antimicrobial usage has changed over time (Lam et al., 2012). One might suggest that this influenced the clinical presentation of *M. haemolytica* in cows over time. Indeed, recent GD data shows that resistance of *M. haemolytica* isolates for tetracyclines in dairy cows is increasing, and for tetracyclines, macrolides and sulfonamides in non-dairy cows.[2] The increase in antibiotic resistance in combination with an increasing reluctance to use antibiotics in an early stage of infection, may be factors driving the increasing occurrence of fatal *M. haemolytica* infections.

Conclusions

The aim of this study was to identify trends in numbers and prevalence of *M. haemolytica* infections in necropsied cattle at GD during the 15-year period from 2004–2018. We found a significant increase in likely fatal *M. haemolytica* infections in necropsied veal calves and dairy cows, but no changes in necropsied dairy calves. The risk factors that contribute to the invasiveness and virulence of *M. haemolytica* need to be clarified. Changes in bacterial virulence factors or in cattle-associated factors (or combinations thereof) may be important in the changes seen in the presentation of fatal infections in cattle in the Netherlands.

Conflict of interest statement

All authors are employees of Royal GD, Deventer, The Netherlands. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.tvjl.2020.105576.

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