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MORBIDITY, MORTALITY AND DRUG USE IN WHITE VEAL CALVES WITH EMPHASIS ON RESPIRATORY DISEASE

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Morbidity, mortality and drug use in white veal calves with emphasis on respiratory disease

Morbiditeit, mortaliteit en antibioticumgebruik bij witvleeskalveren met de nadruk op ademhalingsstoornissen

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For my breathing inspirations: Alien and Robbe

“Measurement is the first step that leads to control and eventually to improvement. If you can’t measure something, you can’t understand it. If you can’t understand it, you can’t control it. If you can’t control it, you can’t improve it.”

H. James Harrington (°1929)

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LIST OF ABBREVIATIONS

ADD	Animal defined daily dose
ADG	Average daily gain
APR	Acute phase response
AR	Antimicrobial resistance
BAV-3	Bovine adenovirus type 3
BB	Belgian Blue
BCV	Bovine coronavirus
BCV-label	Belgian controlled veal
BHV-1	Bovine herpesvirus type 1
BRD	Bovine respiratory disease
BRDC	Bovine respiratory disease complex
BRSV	Bovine respiratory syncytial virus
BVDV	Bovine viral diarrhea virus
CI	Confidence interval
CV	Carcass value
DOF	Days on feed
FPT	Failure of passive transfer
Hb	Hemoglobin
HCW	Hot carcass weight
HF	Holstein Friesian

LIST OF ABBREVIATIONS

HR	Hazard ratio
IgBP's	Immunoglobulin-binding proteins
LKT	Leukotoxin
LOS	Lipo-oligosaccharides
NPS	Nasopharyngeal swab
MbAD	<i>Mycoplasma bovis</i> associated disease
OR	Odds ratio
PDD	Prescribed daily dose
PI	Persistently infected
PI-3	Parainfluenzavirus type 3
R	Range
SD	Standard deviation
SV	Spirometric performance
TI	Treatment incidence
UDD	Used daily dose
Vsps	Variable surface lipoproteins

As animal production became large-scaled and more intensive, producers relied more and more on antimicrobial use as a management tool to overcome disease. Next to pigs and poultry, this is especially true for the veal industry, a niche market in Belgium, specialized in raising male calves on a low iron milk diet. The detection of high levels of antimicrobial resistance in bacteria from livestock raised general awareness on the potential risks for human medicine. Nowadays this concern is overwhelming veterinary medicine and several European countries are eagerly taking measures to reduce antimicrobial use in food animals. With the current political pressure, one even risks of implementing regulations, which are sound to prevent antimicrobial resistance, but economically and ethically unfeasible in the food animal industry.

Evidence based practice aims at combining clinical judgment and expertise with the best scientific evidence, and is the way to walk to rationally reduce antimicrobial use in food animals. To date there is little scientific evidence on successful curative or preventive protocols for the different diseases occurring in white veal calves. This statement can even be extended, since there is hardly any information available on the relative importance of the causes of morbidity and mortality or on the underlying pathogens and their spread in the contemporary European veal industry. This situation has led to empiric, often blind, treatment in the veal industry, frequently applying antimicrobial use as a type of insurance.

The present doctoral thesis aimed at offering insights into the current treatment practices and into the causes and epidemiology of morbidity and mortality in the Belgian white veal industry. Since respiratory disease played a central role, further focus was on characterizing the infectious component of the respiratory disease complex in white veal calves. The obtained results can guide the veal industry in their search for evidence based preventive and therapeutic protocols, reducing antimicrobial use, while maintaining production results and animal welfare standards.

CHAPTER 1

GENERAL INTRODUCTION

THE VEAL INDUSTRY IN BELGIUM AND ITS PAST, PRESENT AND FUTURE CHALLENGES

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THE VEAL INDUSTRY

THE VEAL INDUSTRY WORLDWIDE

In the industrialized world veal meat is a high quality product which has beneficial nutritional values such as a favorable amino acid profile, low fat content and tenderness. It is marketed worldwide and is generally more expensive than beef, pork or poultry. In 2008, European veal consumption stood at 1.6 kg per capita per year with the largest consumers being France (4.1 kg per capita per year) and Italy (3.5 kg year) (Sans and De Fontguyon, 2009). In the European Union (EU), veal is defined as meat from calves aged between 0 and 8 months of age. Since 2008 three effective 'veal' definitions are applicable in Europe (Regulation EC 566/2008). White veal (milk-fed or special fed veal) is white in color (1-10 points on the European color scale) and must be younger than 8 months old at slaughter. White veal is the traditional form of veal production and still holds the largest proportion of the European veal industry. Rosé veal (red, grain-fed or non-formula-fed) also originates from calves younger than 8 months old, but has a more red color (11-14 points) due to the different diet. Meat from bovines aged between 8 and 12 months is locally marketed under different denominations, such as beef (United Kingdom), older rosé veal (Ireland, the Netherlands), Ternera (Spain) or '*jeune bovin*' (France)(Brown and Claxton, 2011).

The veal industry is mainly an important side market of the dairy industry. Since it processes both the surplus of male calves as milk, it plays a major regulating role in the milk and meat industries worldwide (Sans and De Fontguyon, 2009). Of the global veal production in 2010, 82% was produced in Europe. In 2008, European veal production stood at 5.8 million calves, or 806 000 tons of carcass weight (Sans and De Fontguyon, 2009). The main producing countries were France (27%), the Netherlands (25%) and Italy (16%) (Brown and Claxton, 2011). The Belgian veal industry nowadays accounts for 6% of the global production, similar to Germany (5%) (Brown and Claxton, 2011). Veal production in other European countries is limited, as in Switzerland (Bähler et al., 2010), or restricted for welfare reasons in Scandinavian countries. Outside the EU, veal is also produced in the United States (Indiana, Michigan, New York, Ohio, Pennsylvania and Wisconsin) (6% of global production), Canada (4%), Australia (4%) and New Zealand (3%)(Brown and Claxton, 2011). In these countries also bobby calves are

produced (in Australia and New Zealand this is the major form of veal production), which are slaughtered within a week after birth (Cave et al., 2005). In Europe, bobby veal production is present at low scale in Bulgaria and Romania (Sans and De Fontguyon, 2009).

Worldwide the white veal industry is characterized by a high degree of integration, whereas the rosé industry is still private owned (Derks et al., 2005). To provide all these veal herds with calves within the limited time frame possible in an all in/all out production system, a complex network of calf purchase, transport and sorting exists within each country and often extends internationally. Calves, originating from multiple herds are collected by a local tradesman (mostly only a few calves per herd within a certain time frame) and transported to a sorting center, owned by an integration or a larger tradesman. Here the calves are sorted according to their bodyweight and conformation, after which they are transported again to the fattening herd. The typical diet of white veal is an all liquid diet of milk powder. Skimmed milk powder is used, but the protein (casein) component is frequently replaced by cheaper whey or vegetable (soy, pea,...) proteins. The latter product without any animal proteins is referred to as 'nil product'. Milk powder composition can highly differ over time, and the milk diet is often adapted to the calf breed that is used within a certain production system. In most farms milk is distributed to the calves by bucket at start and by drinking trough in the group housing phase. Alternatively, in calves housed in pens of 15 to 70 animals, also automatic milk distributors are used (Sans and De Fontguyon, 2009). In addition to the milk diet, concentrates and roughage are provided.

THE BELGIAN VEAL INDUSTRY

Around the year 1900 male calves were traditionally slaughtered as bobby calves shortly after birth. The veal industry in Belgium started in the regions around Antwerp in close contact with the Dutch veal industry. In these regions the soil consists predominantly out of sand, which is of minor agricultural quality, directing agriculture towards livestock farming. Together with a growing dairy industry an excess of male calves became available. Soon, many producers fattened a number of calves in individual boxes with exclusively excess cow's milk, producing white veal, which was already in those days expensive meat reserved for special occasions. With the invention of skimmed milk powder in 1955 in the Netherlands, the Belgian sector experienced a revolutionary change in the trace of the Dutch sector towards a more industrialized veal production system (Derks et al., 2005). By 1960 veal production already had become the main activity in several farms in the Netherlands (Derks et al., 2005).

Belgium has approximately 2.4 million cattle and approximately 36 000 cattle farms (SANITEL, 2012). Of these, 154 098 are veal calves (Truyen, 2011). The exact number of veal herds in Belgium was only recently determined at 286, of which 96.4% are located in Flanders (Truyen, 2011). More than 70% of the industry is situated in the province of Antwerp (Figure 1). Limburg, Western Flanders, Flemish Brabant and Eastern Flanders account for 13, 10, 5 and 2% of the herds in Flanders, respectively (SANITEL, 2009). The mean herd size is 569 calves, and 92% of the herds are larger than 200 calves (SANITEL, 2009; Truyen, 2011). Belgium produces almost exclusively white veal in three production types which are based on breed. Most herds raise dairy calves (red and black Holstein Friesian (HF) (60%)), but also purebred double muscled Belgian Blue (BB (15%)) calves and crossbreds (predominantly HF x BB (25%)) are present. As in the whole of Europe the sector is highly integrated. There are three main integrators in Belgium with their own milk powder plants and slaughterhouses. Additionally there are another 5 smaller integrators, predominantly specialized in the BB segment. In 2006, 300 036 Belgian veal calves were slaughtered, which accounted for 36.6% of the total number of cattle slaughtered in Belgium in that year (Campers et al., 2008). This number is gradually increasing and reached 321 882 calves in 2010. The heavier BB calves are destined to the domestic market, whereas the lighter calves are exported. In Belgium, veal consumption declined in recent years, reaching 4.1% of the meat consumption per

inhabitant in 2010 (VLAM, 2010). In 2010, 39 456 tons of carcass weight was exported, predominantly to Italy (38.8%), France (22.9%) and Germany (14.7%) (Truyen, 2011). The turnover of the Belgian veal industry is estimated at 600 million euro annually (Truyen, 2011). The Belgian veal industry provides approximately 500 jobs in the veal herds, 400 in milk powder factories and slaughter houses and another 1500 as indirect services (transport, veterinarians and retail) (Truyen, 2011). The three main Belgian integrators are united in the Belgian Society for Veal Producers (BVK- Belgische Vereniging voor Vleeskalverhouders), which introduced the Belgian Controlled Veal Label (BCV-1996) as a horizontal quality assurance system. At present, 98.9% of Belgian veal production is produced under the BCV label (Truyen, 2011). Compliance with the label is certified and controlled by an independent external agency (SGS-Agrilab N.V.).

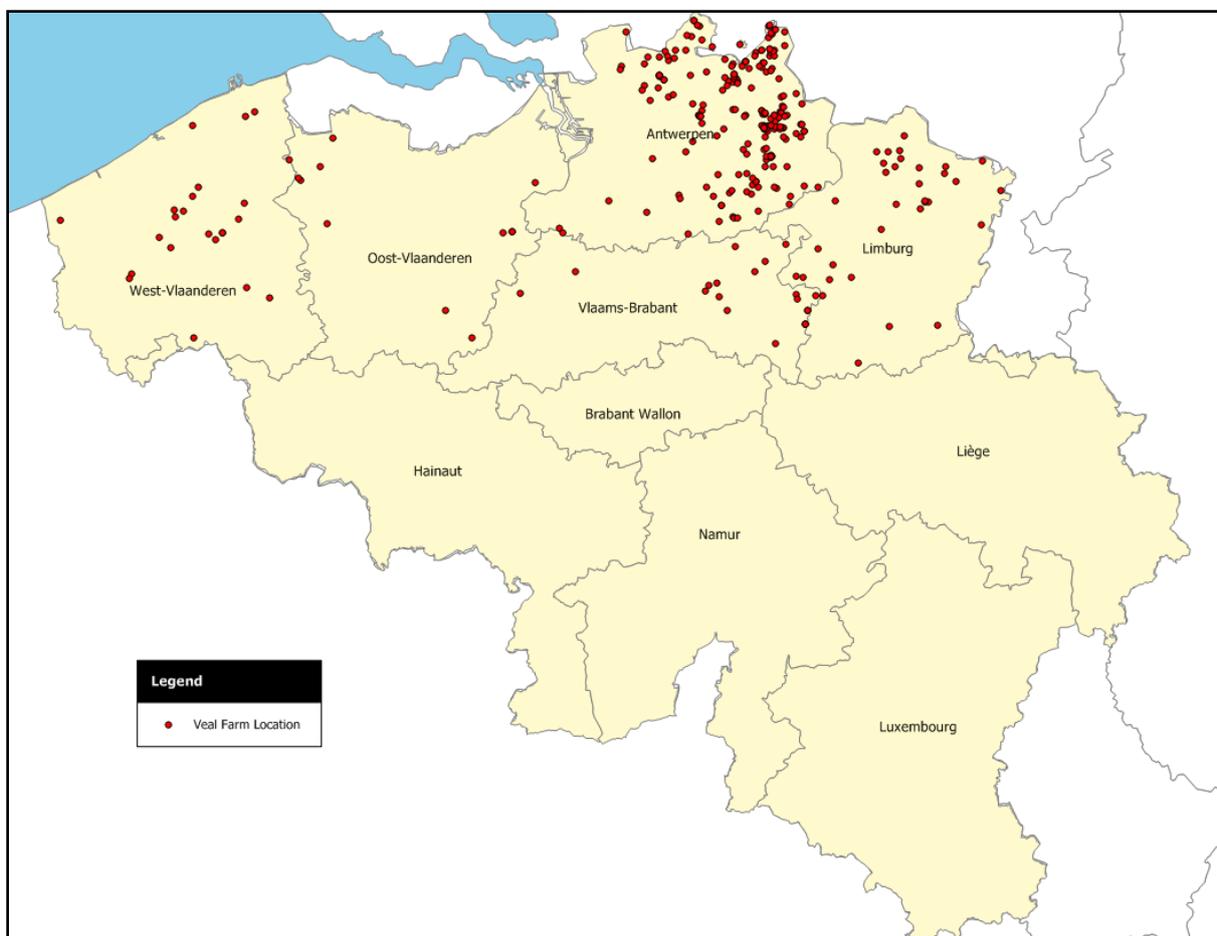


Figure 1. Distribution of veal herds in Flanders as in 2009 (SANITEL-2009) (Figure design: E. Ducheyne).

PAST, PRESENT AND FUTURE CHALLENGES FOR THE VEAL INDUSTRY

The primary consumers demand for white veal is the pale color of the meat. As a consequence, meat color is one of the principal price setters for white veal carcasses. To obtain white meat, veal calves are maintained under specific housing conditions (no access to soil or roughage) and fed specific milk diets to ensure low iron intake. Next to the challenge this already puts to veal producers, the public opinion highly criticizes white veal management and is concerned on the influence that low iron (Fe) levels might have on behavior, welfare and drug use (Wilson et al., 1995). As a consequence the veal industry has been subjected to constant changes in their attempt to maintain the production of high quality veal under changing consumer demands. In the next paragraphs an overview of the most important past, present and future challenges for the veal industry is given.

ANIMAL WELFARE

ANIMAL WELFARE AND THE ALL-LIQUID DIET

The first public remarks on animal welfare in the veal industry date from the 1960's (Derks et al., 2005). The lack of freedom to move, the iron deficiency anemia and the all-liquid diet without any provision of solid feed, which prevents calves from ruminating, were the main issues addressed. Signs of decreased animal welfare are high levels of abnormal behaviors (stereotypes) such as tongue playing, cross-sucking (urine drinking), sucking on the feed trough or coat licking (Bokkers and Koene, 2001). In veal calves also the high incidence of abomasal ulcerations at slaughter is regarded as a sign of reduced animal welfare since abomasal ulcers have been associated with acute and chronic stress or unsuitable feeding strategies (Welchman and Baust, 1987; Wiepkema, 1987; Bähler et al., 2010; Brscic et al., 2011).

A large portion of the animal welfare issues in white veal, as perceived by the public, is situated in the artificial iron anemic state in which the animals are kept. Both the diet and housing conditions necessary to obtain this iron anemia as the potential health consequences are criticized. The red color of meat and blood is caused by the proteins myoglobin and hemoglobin (Hb), respectively. Both molecules have an Fe atom in their molecular structure. By reducing Fe in feed, Hb reduces and carcasses become paler,

without affecting other veal performance and carcass traits (Wilson et al., 1995). However, meat color is affected by much more factors, since Hb only accounted for one third of the variation in visual color score, which makes obtaining the correct meat color a constant challenge for veal producers (Wilson et al., 1995). Hb has been shown to be a better indicator of carcass color compared to plasma Fe (Wensing et al., 1986; Miltenburg et al., 1992a). Normal Hb levels for calves range from 4.9 to 9.3 mmol/L, whereas levels between 4.3 and 4.9 mmol/L are considered marginally anemic (Schwartz, 1990; Wilson et al., 1995). In Belgium, the objectives are 7.7-8.0 mmol/L in the first weeks of production, and 4.9-6.0 mmol/L shortly before slaughter (Personal communication, R. Boone). The principal physiological effect of iron deficiency anemia is reduced appetite, occurring when dietary levels drop below 15 ppm (Webster et al., 1975). A further decrease in iron levels is associated with an impaired immune function, resulting in an increased infection risk (pneumonia), decreased growth performance and an increased feed/gain ratio (Gygax et al., 1993). To meet the public opinion in Europe, dietary Fe intake in veal calves was increased and bound to legal prescriptions (minimum of 4.5 mmol Hb/L (KB 23/1/1998, EC Directives 91/629/EC and 97/2/EC)). Actually, feed efficiency increased without affecting carcass quality when extra iron was supplemented from week 6 on (Miltenburg et al., 1992b). Nowadays, most integrations monitor Hb status several times per production cycle to assure correct carcass color and compliance with European regulations.

Additionally, a gradual uptake of solid feed starting from 50 grams at the age of 8 weeks to a minimum of 250 grams at 20 weeks of age has been obliged (KB 23/1/1998, Council directives 91/629/EC and 97/2/EC). It has been speculated that the provision of solid feed would reduce the quantity of milk powder uptake hereby reducing growth and carcass quality, but not so. Several solid feeds have been tested for their suitability for veal calves. Carcass color was not affected by wheat straw, despite its high Fe content (low bioavailability), whereas for example dried beet pulp evoked too red carcasses (Cozzi et al., 2002). Additionally wheat straw reduced the number of hairballs in the rumen, which is a proxy for abnormal licking behavior (Cozzi et al., 2002). Also abnormal behavior and the time in contact with the feeding trough was decreased in calves provided wheat straw, whereas cross sucking and cortisol curves were not influenced (Mattiello et al., 2002). Unfortunately the provision of wheat straw increased the number of abomasal erosions at slaughter. Recently it has been shown that

especially large amounts of cereal grain are associated with hyperkeratinization and plaque formation in the rumen and abomasal ulceration (Brscic et al., 2011; Prevedello et al., 2012). Creation of a solid feed that improves calves' behavior while maintaining performance and reducing digestive damage, remains a challenge (Mattiello et al., 2002). Reducing the volume of milk provided and increasing the concentration have historically been associated with a reduction in the number of abomasal ulcerations, but this requires confirmation under contemporary management (Welchman and Baust, 1987). Providing drinking water next to the milk diet is not necessary for health reasons, but plays a role in environmental enrichment. Calves consumed almost all of the water that was provided and nonnutritive oral behavior was reduced during production (Gottardo et al., 2002). However, provision of ad libitum water is not advisable as it lead to compulsive drinking (Gottardo et al., 2002).

ANIMAL WELFARE AND HOUSING

The historical rearing system of white veal calves in individual wooden boxes (crates) has been strongly criticized because of poor welfare (Van Putten, 1982; Broom, 1991). In response, several European directives were implemented, guaranteeing minimum space requirements (European directives 91/629/EC and 97/2/EC). From 2007 on group housing for veal calves became obligatory in the European Union. In the United States individual housing is still allowed, but also criticized. Five states already have bans and a complete ban has been advised from 2017 on (Brown and Claxton, 2011). In Europe, calves can still be housed in individual boxes in the first 8 weeks of life (Figure 2). These so called 'babyboxes' are installed in the group housing pen and have fenced lateral partitions allowing social contact with neighbouring calves. At the age of 8 weeks and for the remaining of the production cycle group housing is obligatory with a minimum surface area of 1.8 m² per calf. The most common group housing system applied in Europe is housing on slatted floors in small pens of 4-8 animals. Predominantly in France, calves are raised in larger groups (30-60 calves) and fed by automatic milk delivery devices. In addition, several systems which implement additional animal welfare standards exist in the Netherlands (Peter's farm) and Switzerland (naturafarm) (Bokkers and Koene, 2001; Bähler, 2009a, b). From a welfare perspective, group housing turned out to be more preferable to individual housing allowing social interaction, explorative behavior and more comfortable resting positions

(Le Neindre, 1993; Stull and McDonough, 1994; Andrighetto et al., 1999; Bokkers and Koene, 2001; Babu et al., 2004). However, tongue rolling was only significantly reduced in group housing compared to the smallest individual housing system (0.55 m x 1.50 m) and not when compared with larger boxes (1.10 m x 1.50 m) (Le Neindre, 1993).



Figure 2. Individually housed veal calves in 'babyboxes' on slatted floors in the first 6 weeks of production.

Licking behavior was even increased in group housing compared to the smallest crates (Le Neindre, 1993). Especially the problems of cross sucking (prepuce (urine drinkers), ears, skin,...) and licking of the environment became more pronounced in group housing systems (Le Neindre, 1993; Babu et al., 2004). In group housing cross sucking accounted for 1% of the observed time in 24h time, whereas abnormal oral behavior in total accounted for 21% (Plath et al., 1998). Production results (average daily gain, feed efficiency and dressing percentage) are similar in individual and group housed calves (Andrighetto et al., 1999; Bokkers and Koene, 2001). Calves housed in group housing under Peter's farm conditions (large groups with automatic feeders) showed less oral

behavior, less self-grooming, were lying more and were having less hair balls in the rumen than in individual or conventional group housing, all of which point towards a somewhat improved welfare in the first 6 weeks of production (Bokkers and Koene, 2001). Contradictory, other researchers found the highest amount of sucking activities in herds with automatic delivery devices (Plath et al., 1998). Also, in other production systems, the use of automatic feeder delivery devices was associated with an increased morbidity risk (Maatje et al., 1993; Lundborg et al., 2003; Svensson et al., 2003; Svensson and Liberg, 2006). It is clear that the ideal veal calf housing system, compromising between performance and animal welfare, still needs to be determined. The increasing distance between the life of the European consumer and the reality of farming practices, creates a constant societal concern on animal welfare and a demand for environmental friendly production. To ensure their market, the veal industry constantly needs to address these consumer issues. One possible future perspective is the provision of an outside pen to veal calves, as is already implemented in a high welfare standard veal system in Switzerland (Bähler et al., 2010).

ANTIMICROBIAL RESISTANCE

DEFINITION OF ANTIMICROBIAL RESISTANCE

Antimicrobial resistance (AR) is one of the leading health concerns in human and veterinary medicine worldwide (Hawkey and Jones, 2009). AR can be simply defined as the resistance of a microorganism to an antimicrobial agent. However, reality is more complicated and at present three criteria are used to differentiate between susceptible and resistant bacteria, namely the microbiological, the pharmacological and the clinical criterion. The microbiological criterion implies *in vitro* testing of isolated bacteria for their susceptibility towards different antimicrobial compounds. Frequently used *in vitro* tests are the diffusion (Kirby Bauer disk diffusion, E-test) and serial dilution testing (macro-, microdilution). The minimum inhibitory concentration (MIC), which is the lowest concentration of a certain antimicrobial agent by which growth is visually inhibited, is the unit of measurement to identify resistant isolates. This is done by comparing a strain of interest with wild type strains that are susceptible for a particular antimicrobial agent. Molecular identification of resistance determinants is required to identify the mechanisms and epidemiological relationships. Secondly, the

pharmacological criterion also takes the pharmacokinetics of an antimicrobial in a patient into account. For this criterion the antimicrobial concentration in plasma of healthy individuals is generally used and this has been shown to be a good predictor for the concentration in the infected target tissue (Schentag, 1989; Cars, 1997), although other methodologies in diseased patients can improve accuracy of dosing. Finally, the clinical criterion, which is closest to the field situation, determines the treatment success after administration of an antimicrobial compound directed against a given pathogen. Because of the difficulties in performing and interpreting trials for the latter criterion, the microbiological criterion is most frequently used next to the pharmacological criterion.

AR can be naturally present, due to the fact that the target of the antimicrobial is not present in a given bacterium or an intrinsic resistance gene is present, or can be acquired. A good example of natural AR are *Mycoplasma* species which do not have a cell wall. This implies that penicillins and cephalosporins, two antimicrobial classes which work on the cell wall, cannot work against *Mycoplasma* (Taylor-Robinson and Bebear, 1997). Acquired resistance finds its origin at the molecular level in the occurrence of mutations in the chromosomal genetic material of bacteria or by transfer of genetic material between bacteria (Catry et al., 2003). The latter transfer occurs easily when the genetic material is organized in mobile elements like plasmids (= circular structures of extrachromosomal DNA) (Smet et al., 2009).

Many factors affect AR but the most important trigger of acquired AR is antimicrobial use, which puts a selection pressure on the bacterial populations in a host (both commensal microbiota as pathogens). This association between antimicrobial drug use and the appearance of resistance has been demonstrated under different conditions, including cattle (Tenover and McGowan, 1996; Berge et al., 2006; Donaldson et al., 2006; Jensen et al., 2006; Checkley et al., 2010). Also in certain reports low dosages (underdosing) have been documented as a risk factor for the development of antimicrobial resistance, but in general the importance of underdosing is still questioned (David and Gill, 2008). Another interesting factor is stress, since for example apramycin resistance in faecal commensal bacteria persisted longer in stressed (cold and overcrowded) pigs due to reduced peristaltic dynamics (Mathew et al., 2003). Age is also an important factor since in different production systems calves generally have a

higher prevalence of multiresistant *E. coli* compared to adult cattle (Hoyle et al., 2004; Berge et al., 2005a, 2010). Within the calf period, newborn calves had lower resistance levels compared to 14 and 28 days old calves, indicating that there is a shift towards more resistant bacteria that is not due to antimicrobial pressure (Berge et al., 2005b). Studies on *E. coli* indicated that the vitamin D component in the milk diet might be co-selecting for resistant strains in the digestive tract soon after birth (Khachatryan et al., 2006). As outlined below, dynamics according to age can be different when other bacteria such as nasal methicillin resistant *Staphylococcus aureus* (MRSA) are considered.

In human medicine, treatment failure due to AR of the target bacteria has been demonstrated on numerous occasions (Swartz, 1994; Bronzwaer et al., 2001; Li et al., 2005). Especially the YOPI (young, older, pregnant and immunocompromised patients) group is at risk, due to a reduced activity of the immune system (Li et al., 2005). Despite the fact that most of the acquired AR in humans is likely associated with human antimicrobial use, there is a great concern on the possible transfer of resistant bacteria or resistance genes from animals to humans (Hammerum and Heuer, 2009). This concern is based on the high levels of AR in both opportunistic as pathogenic bacteria from pigs, poultry and veal calves (Catry et al., 2005, 2006; Hendriksen et al., 2008a,b; Persoons et al., 2010). Resistant bacteria or resistance genes can be transferred from animals to humans by direct contact with the animal or its environment or indirectly via contaminated meat (Hammerum and Heuer, 2009; Graveland et al., 2010, 2011; van Cleef et al., 2011). Not only direct transfer of resistant zoonotic agents from animals to humans is feared, but especially the possibility of exchange of resistance genes from animal isolates with the human microbiota (Hammerum and Heuer, 2009). A direct association between animal and human carriage of methicillin resistant *Staphylococcus aureus* (MRSA ST398) has been recently shown in veal calves (Graveland et al., 2010). Also fluoroquinolone resistant *Campylobacter spp.* from poultry have been isolated from human enteritis cases, shortly after the introduction of fluoroquinolone use in veterinary medicine (Endtz et al., 1991). In contrast, other studies estimated the risk that antimicrobial use (e.g. macrolides) in food animals poses for human treatment failure to be very low (Hurd et al., 2004). Based on current knowledge, the precautionary principle as currently applied by the European Union (see ban on antimicrobial growth promoters below) is justified and both reduction and prudent use

of antimicrobials are highly recommended in both human and veterinary medicine. Since the economic consequences of AR in food animals are potentially devastating, prudent antimicrobial use is of great importance for the food animal industry itself next to the potential dangers for human medicine (Watts and Sweeney, 2010).

Antimicrobial compounds are ranked according to their importance for human use (WHO, 2007). Especially development of resistance against the fluoroquinolones, 3rd and 4th generation cephalosporins and macrolides, which are frequently used in veterinary medicine, is highly feared. For several compounds, used in veterinary medicine and ranked as critically important for human medicine, medium to high levels of resistance were found in pigs and poultry (Persoons et al., 2010; Agerso et al., 2011). Fortunately in two pan European surveys the resistance levels towards the newer compounds only used in human medicine were still low in indicator bacteria from poultry, pigs and cattle (including veal) (Bywater et al., 2004; Di Labio et al., 2007; de Jong et al., 2009).

ANTIMICROBIAL RESISTANCE IN VEAL CALVES

To monitor AR in different systems, resistance is mainly studied in commensal enteric indicator bacteria, like *Escherichia coli* and *Enterococcus spp.* (Di Labio et al., 2007). In the Netherlands, the prevalence of multiresistant (resistance to more than one antimicrobial compound) *E. coli* isolates from food animals increased in the last decade (MARAN-2009, 2011). In contrast to pigs and poultry, the prevalence of AR in commensal and pathogenic bacteria has only recently been documented in veal calves. The prevalence of resistant and multiresistant *E. coli* in white veal calves was approximately 68% and 52%, respectively, and has remained stable since 2006 (MARAN-2009, 2011). For comparison, the prevalence of resistant *E. coli* is low in other cattle production systems, for example 18% in conventional dairy cattle as shown in the Dutch surveillance (MARAN-2009, 2011). It is clear that the production system, especially the (medical) management practices within that system, plays a great role in the prevalence of resistance in *E. coli* and other bacteria (Berge et al., 2005a; Carson et al., 2008; Gow et al., 2008a, b; Berge et al., 2010). On large dairy calf raising facilities the application of antimicrobials in milk resulted in a higher prevalence of multiresistant *E. coli* 's compared to no antimicrobial treatment or individually injected antimicrobials (46% vs. 4%) (Berge et al., 2006). Calves individually treated with antimicrobials within 5 days of sampling also had a higher prevalence of resistant *E. coli* compared to

untreated calves (Berge et al., 2005b). The level of resistance observed in *E. coli* isolates was not affected by being housed together with or isolated from treated calves, indicating that there was little or no environmental transfer of resistant traits or bacteria between calves under these conditions (Berge et al., 2005b).

In 2009, *E. coli* isolates from veal calves were most frequently resistant to tetracycline (60%), streptomycin (48%), sulphamethoxazole (45%), ampicillin (41.5%) and trimethoprim (38%) (MARAN-2009, 2011). Resistance to fluoroquinolones and 3rd generation cephalosporins, respectively first and second choice treatment for the important zoonosis *Salmonella*, were lower (18.1% and 1.8-2.3%, respectively) (MARAN-2009, 2011). A recent study showed that after twenty years of quinolone usage in veal calves in the Netherlands, plasmid-mediated quinolone resistance was not widespread, signifying that quinolone resistance is so far limited to clonal distribution, similarly to human medicine (Hordijk et al., 2012). Also in Canada, 70% of the *E. coli* isolates from white veal meat were resistant, with 85 different resistance profiles, of which the most frequent pattern was only resistant against tetracycline (7.4%) (Cook et al., 2011a). Similarly in Swiss veal calves at slaughter resistance levels were 68.7%, 98.7% and 67.8% for *E. coli*, *Enterococcus spp.* and *Campylobacter spp.*, respectively (Di Labio et al., 2007). In Canadian red veal meat *E. coli* resistance levels were somewhat lower, namely 54% (Cook et al., 2011b). In a limited sample from live Belgian veal calves, similarly high levels of resistance in *E. coli* were found, and the percentage of resistant strains differed along the digestive tract within a calf (Catry et al., 2007a).

The finding which had the highest impact on the scientific community and the public opinion was the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) ST398 in white veal calves, reported to be the highest at farm level (88%) of all food producing animals (Graveland et al., 2010). MRSA are bacteria which are resistant against methicillin and in particular the ST398 clone is associated with a large spectrum of AR, including (oxy)tetracycline in 99,9% of the isolates (Cuny et al., 2009; Catry et al., 2010). MRSA poses a serious health risk when involved in common infection of (non)surgical wounds, intravenous catheters or tracheal tubes instead of non-resistant skin flora (Kiyosawa et al., 2000; Roggenkamp et al., 2004), and ST398 has been associated on separate occasions with human infections (Catry et al., 2010). The specific sequence type ST398 of MRSA emerged since 2003 in food producing animals and farmers, and is

nowadays referred to as livestock associated MRSA (LA-MRSA or MRSA ST398) (Tiemersma et al., 2004; Smith et al., 2009). In the Netherlands LA-MRSA could be isolated on 88% of the sampled veal farms (n= 102), and on average in 28% of the calves (nasal carriage) (Graveland et al., 2010). The prevalence of LA-MRSA was (slightly) lower in rosé veal calves than in white veal calves (73% vs. 82%, respectively) (Bos et al., 2012). Significant risk factors for an increased odds of nasal MRSA carriage in calves were increasing age, antimicrobial group treatment, increasing calf density and low farm hygiene (Graveland et al., 2010; Bos et al., 2012). The influence of age on carrier status should be interpreted carefully, since age is strongly associated with changes in housing and feeding and with antimicrobial treatment. Interestingly, in rosé calves the number of days on oral antimicrobials in the arrival treatment increased the odds of MRSA carriage by 1.3 (95% CI: 1.1-1.4) per treatment day, whereas this could not be demonstrated in white veal, likely because of correlation of group treatment with age and lack of variation between farms (Bos et al., 2012). Also performing rodent control was associated with a higher MRSA risk in white veal (Bos et al., 2012). The authors suggest that rodent control results from the presence of rodents due to poor hygiene, and that poor hygiene might cause health issues in calves leading to higher antimicrobial use, but more research is necessary to elucidate this. Factors that were not significantly associated were number of times calves were relocated between pens, presence of other farm animals, presence of multiple stables, sampling month or environmental temperature at sampling (Bos et al., 2012).

In veal producers and their family members MRSA prevalence was 38% and 8%, respectively, whereas this is estimated at 1% in the general Dutch population (Graveland et al., 2010). The risk of being a MRSA carrier increased with increasing number of hours spent per week in the stables, more intense contact with the animals (feeding and veterinary care) and with increasing prevalence of MRSA in the calves (Graveland et al., 2010), in line with other studies (Garcia-Graells et al., 2012). Additionally, it has been shown that even after a short-term contact (max. 3h) with veal calves, 17% of the persons acquired MRSA but in 94% of the cases the strain was no longer detectable after 24h (van Cleef et al., 2011). In contrast to direct contact with animals, the presence of MRSA on meat products is supposed to play a minor role in the transmission of LA-MRSA to humans, provided meat is properly cooked and appropriate hygienic measures are taken (Kluytmans, 2010). The presence of MRSA in these veal

producers and their family is a potential personal health risk if they would experience a wound infection or become hospitalized. Additionally, spread of MRSA from the veal farmers or visitors to a more susceptible population, e.g. hospitalized persons, is an important public health risk, although LA-MRSA has not been shown to spread efficiently between humans (Voss et al., 2005; van Rijen et al., 2008; Wassenberg et al., 2008; van Cleef et al., 2010).

Nowadays, besides MRSA, especially the rise of extended spectrum beta-lactamase (ESBL) producing enterobacteriaceae (e.g. *E. coli* or *Klebsiella spp.*) in food animals is of great concern. ESBL's are enzymes that render Gram-negative bacteria resistant to different beta-lactam classes, including penicillins and cephalosporins. Especially resistance to 3rd and 4th generation cephalosporins, which are classified as critically important for human medicine, is feared (WHO, 2007). ESBL genes are usually located on plasmids, and therefore can be easily spread between enterobacteriaceae even across bacterial genus borders (Coque et al., 2008). Worldwide a 10-20 fold increase in the number of ESBL producing bacteria in hospitals and also among the common population (e.g. urinary tract infections) has been noticed in the last decade (Jacoby and Munoz-Price, 2005; Coque et al., 2008; Forssten et al., 2010; Smet et al., 2010). Tourists and travellers can pick up ESBL gene positive strains and transfer them from one continent to another (Tangden et al., 2010). Because bacterial species that carry ESBL genes are normal inhabitants of the gastrointestinal tract, food is a potential source of them. In this respect, the presence of ESBL genes in 79.8% of the examined poultry meat samples, is of great concern (Overdevest et al., 2011). At present, ESBL is predominantly a poultry issue (44% of the farms in 2007-2008, with the number of positive chickens ranging from 8% to 73% per herd), whereas the prevalence in pigs and veal calves is low (0.46% of the *E. coli* isolates from veal calves in Belgium in the period 2001-2004) (Cattray et al., 2007b; Diarrassouba et al., 2007; Smet et al., 2008; Persoons et al., 2010; Overdevest et al., 2011). In humans, carbapenems are used as a last resort drug to treat infections with ESBL producing microorganisms, but recently also resistance to these drugs due to carbapenemase was found (Evans et al., 2012; Vardakas et al., 2012). Carbapenems are not used in veterinary medicine and so far no resistance to carbapenems has been described in calves.

Also in other major zoonotic bacteria from veal calves the issue of AR is present. Although the prevalence of the zoonotic enterohaemorrhagic *E. coli* (EHEC) and verotoxigenic *E. coli* (VTEC) was low in Belgian veal calves (2.6% and 3.9%, respectively), AR was also abundantly present in these isolates (Bardiau et al., 2010). In this study the well-known human pathogenic EHEC strain O157:H7 was not isolated, but strains belonged to the O26 and O11 serogroups were, posing a potential risk to humans. In Canadian veal calves EHEC O157:H7 was retrieved from 3.2% of the sampled calves, but in contrast these isolates were pansusceptible (Cristancho et al., 2008). Another zoonotic agent, Salmonella, has historically been a major health issue in white veal calves (van Zijderveld et al., 1982; de Visser et al., 1987; Bosch and Hartman, 1993). Outbreaks implying multiresistant strains, including fluoroquinolone resistance, have already been described years ago (Bosch and Hartman, 1993). Faecal Salmonella prevalence in veal calves in older Dutch studies revealed 3.5%, of which 47.5% *S. typhimurium* and 20% *S. Dublin* (van Zijderveld et al., 1982). In more recent Canadian studies, *Salmonella spp.* could still be isolated from 4% of the red veal meat samples and 29% of the strains were resistant (24% multiresistant) (Cook et al., 2011b). Also in Denmark, a minor veal producing country, the true Salmonella prevalence was recently estimated at 34-57% of the veal herds infected with a within herd prevalence of 21-49% (Nielsen et al., 2011). In the only published study from Belgium, no Salmonella was found in 5 farms, studied between 2001 and 2004 (Catry et al., 2007b). Also, in recent years, a very low frequency of Salmonella outbreaks is reported in practice, with little resistance of the isolates (Personal communication, G. Boone).

In recent years, next to the well-known hospital acquired (antimicrobial associated diarrhea) *Clostridium difficile* infection in humans, the incidence of community-associated cases of *C. difficile* infection increased (Goorhuis et al., 2008a; Jhung et al., 2008). Similar strains of *C. difficile* have been isolated from feces of several food animals and have been found in retail meat, predominantly toxinotype V ribotype O78/126 (Rodriguez-Palacios et al., 2006, 2007; Jhung et al., 2008; Songer et al., 2009). Also in recent studies on white veal calves *C. difficile* was highly prevalent shortly after arrival (30% and 36-49% in the first month after arrival in Belgium and Canada, respectively), whereas the prevalence was only 2% shortly before slaughter (Zidaric et al., 2010; Costa et al., 2011). Since both studies were follow up studies on a single farm, this prevalence should be interpreted with care and cannot be seen as representative for the complete

industry. In both studies, as in previous work in conventional calves, ribotype 078 was most prevalent (Zidaric et al., 2010; Costa et al., 2011). This ribotype has been reported as an increasingly important cause of *C. difficile* infection in humans, being the third most common strain in a recent European study and also in Belgium (23.0% of the hospitals) (Goorhuis et al., 2008a,b; Rupnik et al., 2008; Viseur et al., 2011). Despite the lower prevalence just before slaughter, ribotype 078 was most prevalent at that time, posing a potential hazard for meat contamination (Zidaric et al., 2010; Costa et al., 2011). In the Canadian study 76% and 93.1% of isolates was tetracycline resistant shortly after arrival and just before slaughter, respectively (Costa et al., 2011).

Not only the high resistance levels in indicator and zoonotic bacteria are worrisome, but also the resistance levels in major pathogens for the calves themselves. Resistance levels in the most important respiratory pathogens in calves, the Pasteurellaceae, isolated from veal calves are markedly higher than in conventional dairy or beef calves (Härtel et al., 2004; Catry et al., 2005). In a Belgian study, 71.9% of the Pasteurellaceae isolates from veal calves was resistant (Catry et al., 2005). Remarkably, multiresistance was only detected in isolates from veal calves (32.6% of the isolates) and not in dairy or beef calves (Catry et al., 2005). The most frequent resistances for *Pasteurella multocida* were sulphonamides/trimethoprim (93.5%), oxytetracycline (69.5%), tilmicosin (43.4%) and amoxicillin (17.4%) (Catry et al., 2005). For *Mannheimia haemolytica* this were oxytetracycline (100%), amoxicillin (75%), tilmicosin (50%) and sulphonamides/trimethoprim (25%) (Catry et al., 2005). In Swiss veal calves high levels of tylosin (83% and 88% of the tested isolates for *P. multocida* and *M. haemolytica*, respectively) and tilmicosin (56% of *P. multocida* isolates) were noticed (Rérat et al., 2011). However, the latter study aimed at experimentally evaluating different antimicrobial treatments in veal calves. Therefore, these results on AR should be interpreted with care and might not be representative for the field situation. Also a larger within herd variability in resistance profiles of Pasteurellaceae was noticed in veal calves (3-10 different profiles per herd) compared to dairy (1-2 profiles) or beef calves (2-3) (Catry et al., 2005). Resistance to fluoroquinolones was present at low level, whereas no resistance to florfenicol or cephalosporins was detected in that study (Catry et al., 2005). The high levels of AR in respiratory pathogens in veal calves against a wide range of antimicrobial compounds poses a serious risk for the occurrence of treatment

failure for respiratory disease, resulting in production losses and welfare issues in the calves.

CONCLUDING REMARKS

Antimicrobial use in production animals has become a worldwide concern in the face of rising resistance levels, potentially threatening treatment options in human and veterinary medicine (Bywater et al., 2004). Especially in intensive livestock production systems (including veal calves), which frequently use oral antimicrobial group treatments, resistance levels are high (Timmerman et al., 2006; Hendriksen et al., 2008a; Persoons et al., 2010; MARAN-2009, 2011; Persoons et al., 2012). As a consequence, Europe totally banned the use of antimicrobial growth promotors in 2006 (Regulation EC 1831/2003). Unfortunately the trend is, also in Belgium, that these growth promotors are being replaced by preventive (especially premixes) or curative antimicrobial treatments and that total antimicrobial use only slightly decreased (MARAN-2009, 2011; BelVet-Sac, 2012). Europe demands prudent use of antimicrobials both in human as veterinary medicine. In 2005 the European Platform for the Responsible Use of Medicines in Animals (EPRUMA) was established as a multi-stakeholder platform to promote responsible use of medicines in animals in order to maintain efficacy and both prevent and minimize adverse reactions (EC directives 2001/82/EC and 2004/28/EC). In 2012, a Belgian multi-stake holder organization (AMCRA- Antimicrobial Consumption and Resistance in Animals) was initiated to collect and analyze data on antimicrobial consumption and resistance in order to be able to communicate and advise on responsible and prudent use of antimicrobials in food animals. Responsible and prudent use implies only the use of antimicrobials when no other (management) alternatives are available and only when based on sampling results (Ungemach et al., 2006). To obtain this evidence based approach, a thorough knowledge on the causes and epidemiology of all diseases within each livestock production system is of paramount importance. To stress the importance of this issue, the European parliament recently called for a revision of current practices of antimicrobials in veterinary medicine, especially to what concerns prophylactic use (European parliament resolution of 12 May 2011 on antibiotic resistance).

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BOVINE RESPIRATORY DISEASE COMPLEX IN VEAL CALVES

INTRODUCTION

Bovine respiratory disease (BRD) is the major health issue of calves and beef cattle in almost every production system worldwide (Sargeant et al., 1994a, b; Vogel and Parrott, 1994; Edwards, 1996; Sivula et al., 1996b; Busato et al., 1997; Svensson et al., 2003; Assie et al., 2004; Woolums et al., 2005; Caswell et al., 2006; Svensson et al., 2006a,b; Assie et al., 2009a,b; Bähler et al., 2010). BRD increases mortality and treatment costs, reduces growth and carcass weight and causes poor carcass quality, all leading to significant economic losses (Virtala et al., 1996; Gardner et al., 1999; Fulton et al., 2002; Thompson et al., 2006; Schneider et al., 2009; Garcia et al., 2010). The annual costs associated with BRD were estimated at € 95 million in the United Kingdom and at €592 million in the United States feedlot industry (Griffin, 1997; Barrett, 2000). These high costs made BRD management a priority which resulted in numerous publications.

Despite extensive evidence on the infectious nature of BRD, researchers are generally unable to reproduce the most common clinical presentation through experimental exposure to single bacteria and viruses alone (Mohanty et al., 1975; Jericho and Langford, 1978; Castleman et al., 1985; Janosi et al., 2009). On the other hand, the implicated bacterial species can be isolated from the upper respiratory tract (nasopharynx and tonsillar crypts) of healthy cattle and detection in the lung was not consistently associated with clinical symptoms (Allen et al., 1991; Highlander, 2001; Fulton et al., 2002; Autio et al., 2007; Angen et al., 2009). Also, not all calves infected with a respiratory virus under field conditions develop clinical disease (Angen et al., 2009). From this observations it is clear that BRD is a multifactorial disease, resulting from the interaction of viral and bacterial pathogens with calf factors (immunity) and the environment. Therefore contemporary literature uses the terminology bovine respiratory disease complex (BRDC) to stress the multifactorial nature of BRD (Bureau et al., 2001a; Griffin et al., 2010). Usually one or a combination of stressors are needed to initiate BRD (Cusack et al., 2003). These stressors can be viruses, noxious gasses like ammonia, dust, endotoxins, weather conditions (air draft) and stress associated with weaning, transportation or manipulation (Palechek et al., 1987; Arthington et al., 2003; Lundborg et al., 2005; Lomborg et al., 2008). Direct evidence that stress influences the synergy between a viral (in casu BHV-1) and bacterial infection (in casu *M. haemolytica*) leading to more severe lesions in stressed calves is available (Hodgson et al., 2005).

Certain authors make a distinction between “sufficient” components in the BRDC, which are able to cause BRD by themselves, and “necessary but not sufficient” components, requiring additive effects to cause disease (Cusack et al., 2003). However, efforts to discern which factors are overall most important for the initiation of BRD have frequently failed to establish definitive answers (Ribble et al., 1994; Snowden et al., 2005). A possible explanation lies in the fact that the relative contribution of each factor in the BRDC complex differs between production systems and geographical regions, signifying that an essential factor within one production system in a given region, might not be of significant importance in another one. Structural and medical management strongly differs between cattle production systems. Geographical differences can be explained by the absence of certain pathogens in a region (e.g. absence of bovine viral diarrhoea virus (BVDV) and *Mycoplasma bovis* in Finland (Härtel et al., 2004; Nikunen et al., 2007)), by a different climate (Sivula et al., 1996a; Assie et al., 2004;) and by socioeconomic factors related to the producers (van der Fels-Klerx et al., 2011). But even repetitive studies in the same feedlots over several years faced highly variable outcomes and at present, it is even questioned whether BRD is contagious, clusters according to risk factors or occurs randomly (Taylor et al., 2010b). One large, multi-year study in preweaned beef calves found little temporal and spatial clustering effects, suggesting that BRD is not contagious and that risk factors are hard to identify (Snowden et al., 2005). Another study did detect clustering within source and pen, demonstrating that BRD is not random and therefore suitable for epidemiological research (Ribble et al., 1994). This issue is further addressed in studies on the molecular epidemiology of Pasteurellaceae strains (Allen et al., 1992; Highlander, 2001; Hotchkiss et al., 2010; Klima et al., 2011).

The complexity of BRD demands knowledge on the BRDC in each production system to be able to determine effective control measures for that particular production system. Whereas the BRDC is extensively studied in feedlots and conventional (large and small scale) dairy calf rearing, there are few studies available documenting the BRDC in veal calves. Therefore, in the next paragraphs, an overview of the constitution of the BRDC in veal calves (calf factors (immunity), the infectious component and environmental influences) is given based on the available literature for veal calves and extrapolation from other production systems.

IMMUNITY AND OTHER CALF FACTORS

On arrival, veal calves experience an increased exposure to pathogens, environmental, social and dietary changes. In order to cope with these challenges an adequately working immune system is essential. Immunity is generally classified as innate (natural or aspecific) or acquired (specific) (Galyean et al., 1999). Innate immunity consists of physical and chemical barriers, the acute phase response (APR) and phagocytes (macrophages, neutrophils and natural killer cells) (Galyean et al., 1999). Respiratory epithelium in the nasal cavity, trachea and principal bronchi has several innate protection mechanisms to clear the lower airways from pathogens. Mucus prevents adherence of the bacteria and cilia remove the pathogen within the mucus through an upwards movement. In addition bactericidal peptides such as surfactant proteins A and D and defensins adhere to microorganisms, opsonizing them for phagocytes (Ackermann et al., 2010). Healthy calves are able to clear 90% of an inhaled dose of bacteria within 4 hours (Lillie and Thomson, 1972). Together with phagocytes the APR forms the first line of defense. The APR consists of the increased synthesis of a large series of proteins which have opsonizing, binding, coagulative, fibrinolytic or chemotactic functions aiding in the process of inflammation as a defense mechanism of the body against foreign substances. Of these proteins complement, C-reactive protein, haptoglobin, serum amyloid A, fibrinogen and ceruloplasmin have most frequently been studied as markers of inflammation in cattle (Humblet et al., 2004; Nikunen et al., 2007). Acquired immunity occurs at a later stage after natural exposure or vaccination and is divided into humoral and cell-mediated immunity. Humoral immunity implies the production of immunoglobulins (IgM, IgG1, IgG2, IgA and IgE) by B- lymphocytes, which provides defense against extracellular microbial infections (Galyean et al., 1999). Additionally, cell-mediated immunity provided by T-lymphocytes protects against intracellular pathogens and tumor cells (Galyean et al., 1999).

Calves are born with a functional immune system and are capable of responding to certain antigenic stimuli, but the system does not yet operate at the optimum response capacity (Mallard et al., 1998). At birth, calves have very low immunoglobulin levels because of the epitheliochorial placenta in ruminants, which does not allow passage of maternal antibodies when intact (Kruse, 1983; Latshaw, 1987). Therefore calves are totally dependent on the passive transfer of immunoglobulins from the dam via

colostrum. The primary immunoglobulin in bovine colostrum is IgG1 (85%), which is derived from maternal serum IgG1 (Larson, 1958; Murphy et al., 1964; Pierce and Feinstein, 1965; Sasaki et al., 1976; Barrington et al., 1997). Failure of passive transfer (FPT) occurs when an inadequate quantity of immunoglobulins has been transferred to the calf. The presence of FPT is most frequently determined by measuring the IgG levels or less accurate the total protein levels in healthy calves aged 2-8 days (McDonough et al., 1994; Donovan et al., 1998a;). Total protein and IgG concentrations lower than 55 g/L and 10 g/L, respectively, are generally considered as FPT (McDonough et al., 1994; Donovan et al., 1998a; Wilson et al., 2000; Beam et al., 2009).

The prevalence of FPT is rather high in white veal calves with large regional differences. The prevalence of FPT in veal calves was 80% in Western US, ranged between 20 and 40% in Eastern US and was 38% in Canadian veal calves (McDonough et al., 1994; Wilson et al., 1994, 2000; Stilwell and Carvalho, 2011). Although the consequences of FPT were studied in European veal calves, the prevalence of FPT in veal has not been reported in Europe as such (Postema and Mol, 1984; Postema et al., 1987). Overall, FPT has been associated with an increased mortality risk, with diarrhea and with respiratory disease (Postema and Mol, 1984; Furman-Fratczak et al., 2011). The mortality risk increased markedly when total protein was lower than 50 g/L (Braun and Tennant, 1983; Donovan et al., 1998a). In large scale dairy calf rearing FPT continued to influence mortality until 6 months of age (Donovan et al., 1998a). Studies in conventional dairy calves that included disease history did only find a significant effect of FPT on daily growth in the first month of life or no significant relationship, when accounting for disease occurrence (Virtala et al., 1996; Donovan et al., 1998b). Studies that evaluated relationships between FPT and disease occurrence in veal calves are scarce. Two older studies by the same investigators, conducted in Dutch veal farms with individual housing, had conflicting results. In one study on 65 veal calves an increased risk for BRD was demonstrated in calves with low IgG levels at arrival, whereas in a later study on 78 calves there was no significant association (Postema and Mol, 1984; Postema et al., 1987). Maternal antibodies gradually decline and the seronegative status is reached between 122 and 192 days of age for most respiratory viruses involved in BRD (Fulton et al., 2004). Endogenic antibody production by the calf starts shortly after birth and overcomes maternal immunity at the approximate age of 1 month (Hässig et al., 2007). This gradual decline of maternal immunity (e.g. half-life of antibodies directed against

respiratory viruses ranges from 21 to 36 days) and increase of endogenic antibodies leaves an immunity gap between 2 and 6 weeks of age in which calves might have too low antibody levels to overcome large infection pressures (Fulton et al., 2004). More recently it has been shown that next to immunoglobulins, also colostral leukocytes and proinflammatory cytokines are transferred to the calf. Maternal colostral leukocytes have been shown to accelerate fetal lymphocyte development, whereas the colostral cytokines trigger neutrophil function (Roth et al., 2001; Ackermann et al., 2010). Both factors are likely to have a systemic protective effect, enhancing the calves ability to cope with infections.

Next to immunity other calf factors affect the BRD risk. There are conflicting results in literature, but generally male gender has been associated with an increased BRD risk in beef calves, dairy calf rearing and feedlots (Mugglicockett et al., 1992; Gallo and Berg, 1995; Wittum and Perino, 1995; Snowden et al., 2006). This is of importance since veal calves are predominantly male calves from the dairy industry. Also calves housed in mixed gender pens are at greater risk (Sanderson et al., 2008). Why male calves are at greater risk for BRD is not fully understood. Male calves also have a greater mortality risk, potentially related to this increased BRD risk (Cusack et al., 2007; Bähler et al., 2010). Also the temperament of the calf might play a role, since nervous calves were more likely to be treated for BRD (Fell et al., 1999). Breed differences have been documented in several studies. Herefords and Aberdeen Angus calves were more susceptible to BRD compared to Charolais, Blonde d'Aquitaine, Simmental and crossbreeds (Snowden et al., 2005; Hägglund et al., 2007). An older study reported a higher susceptibility of Pinzgauer calves compared to Hereford, Simmental and Gelbvieh and of Braunvieh compared to Charolais and Limousin (Mugglicockett et al., 1992). Also double muscled Belgian Blue calves are highly susceptible to BRD (Bureau et al., 2001a,b). Beneficial effects of heterosis were suggested in several studies, but only one study could demonstrate a significantly decreased BRD incidence in crossbred compared to purebred British cattle (Mugglicockett et al., 1992; Snowden et al., 2005, 2006). Heritability of BRD susceptibility, as for other fitness traits, appears to be low ($h^2 = 0-0.26$) (Mugglicockett et al., 1992; Snowden et al., 2005, 2006). Birth weight is one of the parameters with the largest genetic correlation with BRD susceptibility, making heavy calves at birth more susceptible (Mugglicockett et al., 1992). Also in Belgian Blue calves a correlation between high spirometric performance (SV) and low economic cost

related to BRD exists (Bureau et al., 2001a). Heritability's for SV were higher ($h^2 = 0.28-0.44$) and SV was correlated with other positive production traits such as body weight and muscling score, making SV a possible tool for selection programs (Bureau et al., 2001b).

PATHOGENS

Most pathogens involved in BRD have been extensively studied. The present article aims at providing a limited overview of each pathogen, for the reader to understand the pathogenesis of BRD in veal calves. *Arcanobacterium pyogenes* (recently renamed as *Trueperella pyogenes* (Yassin et al., 2011)), will not be further addressed, since its role is limited to chronic, abscessing pneumonia. Also certain viruses (Bovine calicivirus, Bovine Rheovirus, Bovine Enterovirus, Bovine Rhinovirus, Bovine Parvovirus and Bovine Herpesvirus type 4) and *Chlamydia spp.*, which could so far not be unequivocally associated with BRD, are outside the scope of the present article (Reggiardo et al., 1989).

VIRUSES

The viral component of the BRDC consists of bovine respiratory syncytial virus (BRSV), parainfluenzavirus type 3 (PI-3), bovine herpesvirus type 1 (BHV-1), bovine viral diarrhea virus (BVDV), bovine coronavirus (BCV) and bovine adenoviruses (BAV). Generally, viruses damage the respiratory epithelium and compromise immune function, enabling secondary bacterial invasion.

BOVINE RESPIRATORY SYNCYTIAL VIRUS

BRSV (Family Paramyxoviridae; genus Pneumovirus) is the most documented respiratory virus in cattle, not only because of its close association with human respiratory syncytial virus, but certainly because of the high incidence worldwide and its potential to cause severe pneumonia as such with mortality risks of on average 2-3%, reaching up to 25% (Bryson et al., 1978b; Holzhauer, 1979; Kimman et al., 1988; Oberst et al., 1993). BRSV is one of the most common diseases in the first year of life in cattle, with highest incidences in autumn and winter (Van der Poel et al., 1993). Calves are infected through inhalation of small-particle aerosols (Van der Poel et al., 1994). BRSV primarily targets the respiratory epithelium of nasopharynx, bronchi, bronchioli and alveolar spaces, causing loss of cilia and necrosis (Aherne et al., 1970). It is speculated

that direct cytopathology due to virus replication is of minor importance, whereas the host response to the virus infection is responsible for the majority of the damage (Aherne et al., 1970; Zhang et al., 2002; Antonis et al., 2010). The typical picture of BRSV associated pneumonia at necropsy consists of cranioventral pneumonia in which the virus can be detected and a caudodorsal zone of congestion and edema (interstitial pneumonia) in which the virus is not present (Kimman et al., 1989a). Microscopic lesions in the cranioventral area include bronchial and bronchiolar epithelial necrosis, and typically multinucleated (syncytial) cells are seen with bronchitis and bronchiolitis (Kimman et al., 1989a). It was suggested that the edema and congestion in the caudodorsal zone, which is responsible for rapid hypoxia and severe symptoms, is due to an immunopathology (activation of mast cells and liberation of histamine) (Kimman et al., 1989b). This is attributed to the fact that in some individuals anti-BRSV IgE antibodies are produced, leading to more severe pathology (Gershwin et al., 2011). In severe cases, interlobular and bullous emphysema are typical findings of BRSV infection, in contrast to other viruses.

BRSV typically runs a more severe course in animals aged between 1-6 months old, whereas newborns (up to 1 month) are generally not severely affected (Bryson et al., 1978a; Kimman et al., 1987, 1988). Although it was believed for years that this difference was due to the presence of maternal antibodies in calves aged under 1 month, this was recently contradicted (Kimman et al., 1987; Antonis et al., 2010). Colostrum deprived neonates hardly showed any symptoms after experimental infection, whereas they shedded the virus for a longer time and had increased lung pathology at necropsy compared to 6 weeks old calves that also did not receive colostrum at birth (Antonis et al., 2010). The possible influence of maternal antibodies on the disease outcome remains a controversial issue, since maternal antibodies (predominantly IgG1) offer incomplete protection to BRSV infection and even low levels of maternal antibodies largely suppress local and systemic antibody responses (Kimman et al., 1987; Antonis et al., 2010). A difference in immunocompetence (immunological immaturity in neonates), rather than the presence of maternal antibodies, is suggested to explain the observed age difference in symptomatology (Antonis et al., 2010). In North American beef calves, the half-life of maternal antibodies against BRSV in non-vaccinated calves was 35.9 days and the mean time to seronegative status was 186.7 days (Fulton et al., 2004). Local IgA plays a more

important role in the protection of calves against BRSV than IgG1 in the lung, which is derived from the blood (Kimman et al., 1987).

It is not well understood were BRSV persists in a population of cattle for the virus to survive. Reinfections of cattle with BRSV have been described most frequently in young animals, but also in adults (Martin, 1983; Van der Poel et al., 1993). These infections result in a strong and rapid secondary immune response (IgA) and usually remain subclinical (Kimman et al., 1987). Persistently infected calves or adults might exist, with reshedding after environmental stressors as a consequence (Baker et al., 1986b). However, at present the most likely explanation for recurrent infection in a herd is thought to be reintroduction of the virus, since high genetic diversity was found between strains from recurrent outbreaks (Larsen et al., 2000).

Limited information on the prevalence and importance of BRSV in the BRDC of veal calves is available. In French group housed veal calves, seroconversion against BRSV was detected in 6 out of 9 herds (66.7%), with a within herd prevalence of 22.2% ranging from 7 to 40% (Arcangioli et al., 2008). The virus could only be isolated from one calf without clinical symptoms in that study.

PARAINFLUENZAVIRUS TYPE 3

PI-3 (Paramyxoviridae; genus *Respirovirus*) is endemic worldwide and the associated disease is named shipping fever, because of the first isolation of PI-3 from recently shipped cattle in the United States (Andrewes et al., 1959). Like other respiratory viruses PI-3 spreads primarily by large droplet transmission and aerosol inhalation. The virus infects nasopharyngeal, tracheal cells, bronchial and bronchiolar cells, type I and II pneumocytes and pulmonary alveolar macrophages (Tsai and Thomson, 1975; Bryson et al., 1983). Infection causes loss of cilia, intracytoplasmic inclusion bodies, swelling and desquamation of epithelial cells, eventually leading to epithelial proliferation (Campbell et al., 1969; Tsai and Thomson, 1975; Bryson et al., 1983). Infection of macrophages results in decreased cytotoxicity for virus infected cells, depression of phagocytosis and bacterial killing, and an altered arachidonic acid metabolism resulting in the secretion of immunosuppressive prostaglandins (Liggitt et al., 1985; Laegreid et al., 1989). Although clinical signs are dose dependent under experimental conditions, most uncomplicated PI-3 infections are mild with coughing, serous nasal discharge and fever followed by

recovery within 10 days (Marshall and Frank, 1971, 1973). Subclinical infections have been reported (Woods, 1968). Intranasal inoculation of PI-3 led to local (IgA) and systemic (IgM and IgG) responses, which were detectable 6 days post infection and persisted for 6-8 weeks and 3-5 months, respectively (Marshall and Frank, 1971). Reinfection led to persistence of local antibodies for 5 months and systemic antibodies even longer (Marshall and Frank, 1971). Whereas local antibodies are regarded most important for the prevention of infection, systemic antibody levels play a role in reducing the severity of the disease once it occurs (Gates et al., 1970; Marshall and Frank, 1971). Persistence of PI-3 maternal antibodies was found to be directly related to the initial concentration transferred (Dawson, 1966). The half-life of maternal antibodies directed to PI-3 was 30.3 days and the mean time until seronegative status was 190.6 days in North American beef calves (Fulton et al., 2004). Another study reported that the average time of antibody decay to susceptible levels was about 10 weeks (Dawson, 1966). Although, colostrum fed calves can be experimentally infected, viral shedding and clinical symptoms were less severe compared to colostrum deprived calves (Marshall and Frank, 1975).

There is very little information on the present epidemiologic situation with regard to PI-3 worldwide. Also in veal calves, there is little knowledge on the prevalence and importance of PI-3 in the BRDC. In French group housed veal calves, seroconversion against PI-3 was detected in every studied herd (n= 9) affecting 23% of the calves on average, ranging from 7 to 53% (Arcangioli et al., 2008).

BOVINE HERPESVIRUS 1

BHV-1 (Family Herpesviridae, subfamily Alphaherpesvirinae) is associated with infectious bovine rhinotracheitis (IBR), abortion, infectious pustular vulvovaginitis (IPV) and systemic infection in neonates (Metzler et al., 1985; van Oirschot et al., 1995; Muylkens et al., 2007). BHV-1 was classified into three subtypes, based on their predisposition for mucosae (genital or respiratory). This classification was recently contradicted since both genital and respiratory strains were able to infect respiratory and genital mucosae (Steukers et al., 2011). BHV-1 is preferentially transferred through nose to nose contact, but also airborne transmission over short distances has been demonstrated (Mars et al., 2000). BHV-1 infects epithelium (respiratory, genital, intestinal,...), peripheral blood mononuclear cells, endothelium, neurons and fibroblasts

(Muylkens et al., 2007). BHV-1 infections are usually limited to the upper airways (nasal cavity and trachea), but infection of pneumocytes has been demonstrated under experimental conditions, when coinfecting with BVDV (Potgieter et al., 1984a). The severity of BHV-1 associated disease depends on the virulence of the strain, host factors and potential bacterial superinfection (Kaashoek et al., 1996). The virus alone can induce a potentially fatal disease (IBR), of which the symptoms are high fever, anorexia, coughing, excessive salivation, nasal discharge, conjunctivitis with lacrimal discharge, inflamed nares and dyspnea (Muylkens et al., 2007). On the other hand many BHV-1 infections are subclinical (Muylkens et al., 2007).

Next to the typical clinical picture of IBR, BHV-1 can present as one of the factors of the BRDC, by promoting bacterial superinfections. This is done by damaging respiratory epithelium (reducing mucosal clearance) and especially by inducing immunosuppression (diminishing the activities of alveolar macrophages and neutrophils and causing selective depletion of CD4+ populations) (Griebel et al., 1987, 1990; Tikoo et al., 1995; Warren et al., 1996; Winkler et al., 1999; Hodgson et al., 2005; Leite et al., 2005). This BHV-1 associated immunosuppression is short lived since cattle mount a potent immune response eventually during acute infection, with neutralizing antibodies appearing 8-12 days post infection (Rouse and Babiuk, 1978). This antibody response is of critical importance in preventing secondary infections and limiting the consequences of reactivation (Babiuk et al., 1996). Regardless of the involvement of secondary bacterial infection, BHV-1 establishes lifelong latency in ganglia following acute infection (Muylkens et al., 2007). Stress and other stimuli can lead to reactivation and viral re-excretion, and this is thought to be the primary mechanism of the virus to maintain within a cattle herd (Muylkens et al., 2007). The mean half-life of maternal antibodies is 21.2 days, and seronegative status is reached after 122.9 days on average (Fulton et al., 2004). Colostral immunity protects neonates from clinical symptoms after infection (Mechor et al., 1987; Lemaire et al., 2000).

BHV-1 has a worldwide distribution although some countries have a long history of BHV-1 control and have successfully eradicated the virus (e.g. Scandinavian countries, Austria and Switzerland). The trading restrictions from endemic to free regions are of the greatest economic importance, being nowadays even more important than the losses associated with the disease itself. Therefore, many European countries, including

Belgium, have eradication programs running. The prevalence and relative contribution of BHV-1 to the BRDC complex in veal calves is currently unknown.

BOVINE VIRAL DIARRHEA VIRUS

BVDV (Family Flavivirus, genus Pestivirus) is the cause of bovine viral diarrhea (BVD), which involves a wide range of clinical presentations, depending on the virulence of the strain, the type of infection (transient or persistent), time of exposure (pre- or postnatal) and the interaction with other pathogens (Ridpath, 2010). Two genotypes (I and II) and two biotypes (non-cytopathogenic and cytopathogenic) are distinguished (Ridpath, 2010). Crucial in the epidemiology of BVDV are calves, born persistently infected (PI) with BVDV, which permanently excrete non-cytopathogenic virus to their environment. Next to the presence of congenital anomalies, PI calves can develop a highly fatal form of BVD, namely mucosal disease (BVD-MD). The role of BVDV in BRD is well studied, but still not fully elucidated, certainly not its total (economic) impact. In feedlots PI calves were more likely than the general population to be treated for BRD and either became chronically ill or died (Loneragan et al., 2005; Ridpath, 2010). Harder to estimate is the importance of a transient (acute) BVDV infection in the BRDC. BVD is a systemic infection, with the nasal mucosa as the primary site, from where lymphocytes in the local draining lymph nodes are infected. These infected lymphocytes spread to other tissues in the viremic phase. Under experimental conditions, infection with most strains of BVDV alone typically leads to mild or moderate respiratory disease (Potgieter et al., 1984b; Brodersen and Kelling, 1998; Baule et al., 2001). Differences in pneumopathogenicity of BVDV strains have been documented, involving both cytopathic and non-cytopathic strains (Potgieter et al., 1985; Jewett et al., 1990). Infection causes lymphoid depletion (cell death and reduced function of remaining lymphocytes) and the resulting immunosuppression is believed to be the most important contribution of BVDV to BRD (Ridpath et al., 2000; Ridpath et al., 2007). Nevertheless, despite this immunosuppression and the occurrence of isolated outbreaks with hypervirulent strains, it is important to notice that between 70 and 90% of BVDV infections are subclinical (Ames, 1986). A synergy between BVDV and most respiratory pathogens (BRSV, PI-3, BHV-1, *Mannheimia haemolytica* and *Mycoplasma bovis*) exists, resulting in an enhanced or changed pathogenesis (more severe clinical signs), most frequently leading to increased dissemination of pathogens in tissues (Potgieter et al., 1984a;

Brodersen and Kelling, 1998; Elvander et al., 1998; Haines et al., 2001; Shahriar et al., 2002; Aly et al., 2003; Ganheim et al., 2003, 2005). These concurrent infections occur frequently, since BVDV was most frequently involved in multiple viral infections (Richer et al., 1988; Fulton et al., 2000). Transient BVDV infections in the BRDC are important, since BVDV could be isolated from 29% of the fatal BRD cases in Canadian feedlot steers, whereas only 0.3% was persistently infected (Booker et al., 2008). Also seroepidemiological studies in feedlots demonstrated associations between seroconversion against BVDV and increased risks for BRD (Martin et al., 1999; O'Connor et al., 2001). Maternal antibodies against BVDV are protective and have a half-life of 23 days on average (Fulton et al., 2004). Seronegativity is reached approximately at 180 days of age (Fulton et al., 2004).

BVDV is an economically important disease worldwide, and several European countries have initiated BVDV control or eradication programs, with the Scandinavian countries having nearly eliminated BVDV in their cattle population (Greiser-Wilke et al., 2003; Van Campen, 2010). Elimination of PI animals is key for a successful BVDV control program, and with the availability of ear notch testing, hope is to eliminate BVDV in Belgium as well. In a North American study focusing on pathogens associated with diarrhea in veal calves, BVDV could be isolated from 6.6% of the calves with diarrhea, all of which had repeated bouts of illness (McDonough et al., 1994). In France, the presence of BVDV was confirmed in 88.8% (8/9) of the examined veal herds (Arcangioli et al., 2008). In that study transiently and persistently infected animals were detected on 66.7% and 44.4% of the herds, respectively. The prevalence of PI animals in the Belgian veal industry is unknown, but in the general Belgian bovine population, 0.3% of the animals aged between 6 and 12 months is PI (Sarrazin et al., 2012).

BOVINE CORONAVIRUS

BCV (Family Coronaviridae, genus Coronavirus) infects both respiratory (from the nasopharynx to alveolar cells) and intestinal epithelium (Storz et al., 1996; Hasoksuz et al., 2002). As a consequence BCV is associated with three distinct syndromes in cattle, and despite the presence of antigenic variation between isolates, all subtypes of BCV can be isolated from all three syndromes (Clark, 1993; Tsunemitsu and Saif, 1995; Hasoksuz et al., 1999a). The role of BCV in neonatal calf diarrhea has been well documented (Clark, 1993). Less frequently BCV causes haemorrhagic diarrhea (winter dysentery) in adult cattle (Tsunemitsu et al., 1995; Cho et al., 2000). Although its role in the BRDC complex of young cattle has been doubted for years, more recent work, predominantly in North American feedlots, has made clear that BCV contributes to the BRDC (Storz et al., 1996; Martin et al., 1998; Hasoksuz et al., 1999b; Hasoksuz et al., 2002; Fulton et al., 2011). Recently, in Italy, the involvement of BCV next to other pathogens in respiratory disease outbreaks in 2-3 month old calves has been demonstrated and in 2 out of 4 outbreaks diarrhea was present at the same time (Decaro et al., 2008a). Whereas experimental infection of young calves resulted in diarrhea in all calves, respiratory BCV infection without involvement of other pathogens is usually associated with mild respiratory disease (coughing, serous nasal discharge, mild fever) (Saif et al., 1986). Concurrent faecal and nasal shedding occurred in 38% of infected calves (Hasoksuz et al., 2002). There have also been severe outbreaks of respiratory disease and diarrhea reported, which were solely associated with BCV (Decaro et al., 2008b).

BCV is ubiquitous, affecting calves in different production systems worldwide (Ganaba et al., 1995; Härtel et al., 2004; Hägglund et al., 2006; Fulton et al., 2011). Information on BCV in veal calves is limited to a single study assessing pathogens associated with diarrhea in the first month after arrival (McDonough et al., 1994). BCV could be isolated from 29.7% of calves with diarrhea in that period. The prevalence and relative contribution of BCV to the BRDC complex in veal calves is currently unknown.

BOVINE ADENOVIRUS

Worldwide, 10 serotypes (1-10) of bovine adenoviruses (BAV) (Family Adenoviridae, genus Atadenovirus) are recognized (Benko et al., 2000). In human medicine, adenoviruses are known to be important pathogens in immunocompromised hosts, such as neonatal infants (Zahradnik et al., 1980). Also in neonatal calves, cases of fibrinous enteritis, sometimes with concurrent pneumonia, associated with BAV-4 and 7 have been reported (Reed et al., 1978; Scanziani et al., 1989; Giusti et al., 1998). The role of adenoviruses in the BRDC remains controversial. Some serological studies supported a role for BAV (Lehmkuhl et al., 1979; Baker et al., 1986a; Mattson et al., 1988; Caldow et al., 1993), whereas others did not find an association (Stott et al., 1980; Baker et al., 1986b). Vaccinating calves against serotype 3 in a problem herd, resulted in a lower prevalence of pneumonia, supporting an initiating role of BAV-3 (Mattson et al., 1987). In calves, serotypes 3 and 5 appear more pathogenic than others, causing respiratory and gastrointestinal disease (Darbyshire et al., 1966; Darbyshire et al., 1969; Ide et al., 1969). BAV infects both respiratory epithelium as macrophages, reducing phagocytosis and killing abilities, and thereby suppressing the immune system of the host (Adair et al., 1992). The respiratory form of BAV-3 infection has been experimentally reproduced by endobronchial inoculation in both colostrum deprived calves as in calves which received colostrum (Darbyshire et al., 1966; Yamada et al., 2003). Interestingly, the lesions in 1 week old calves involved bronchitis, bronchiolitis and alveolitis with intranuclear inclusion bodies, whereas there were no lesions in 5 week old calves. Immunosuppression, experimentally induced by dexamethasone administration, caused again severe lesions in 6 week old calves, comparable with those in newborns (Yamada et al., 2003). A difference in immunocompetence between neonates and older calves, is held responsible for the more severe lesions in younger calves (Narita et al., 2002). Together with BVDV, BAV is frequently involved in multiple viral infections (Richer et al., 1988).

BAV infections occur worldwide. The current situation in Europe is hardly known with exception of some Nordic countries. Based on these reports both BAV-3 and 7 are widespread, affecting 50-100% of Finnish herds and between 19 to 72% of the calves (Härtel et al., 2004; Autio et al., 2007; Nikunen et al., 2007). The prevalence and relative contribution of BAV to the BRDC complex in veal calves is currently unknown.

BACTERIA

Respiratory bacteria associated with BRD belong to the Pasteurellaceae or Mycoplasma family.

PASTEURELLACEAE

The *Pasteurellaceae* spp. involved in BRD are *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* (Griffin, 2010; Griffin et al., 2010). Pasteurellaceae are small Gram-negative, facultative anaerobic bacteria which are present in the upper airways as well as in the tonsillar crypts of healthy and diseased animals (Allen et al., 1991; Highlander, 2001; Catry et al., 2005). *M. haemolytica* is considered to be the predominant bacterial pathogen associated with BRD (Griffin et al., 2010). *Mannheimia haemolytica sensu lato* involves 5 species, namely *M. haemolytica sensu strictu*, *M. granulomatis*, *M. glucosida*, *M. ruminalis* and *M. varigena* (Biberstein et al., 1960; Christensen et al., 2004). *M. haemolytica* has 12 capsular serotypes (A1 and A6 are most prevalent in cattle, A1 highly pathogenic and A2 non-pathogenic) and several virulence factors (Highlander, 2001; Griffin, 2010). They have a capsula, produce lipopolysaccharide (= endotoxin), an exotoxin (= leukotoxin (LKT)), adhesion proteins (e.g. outer membrane proteins (iron binding proteins)), produce enzymes (neuraminidase and sialoglycoprotease) and a biofilm (Pancieria and Confer, 2010). The most important virulence factor is LKT. Low concentration of LKT induces apoptosis in leukocytes while stimulating the release of reactive radicals, causing severe inflammation of the lung (Czuprynski et al., 2004; Zecchinon et al., 2005; Rice et al., 2007). Higher concentrations totally impair immune function and cause necrosis (Czuprynski et al., 2004). It is believed that these virulence factors enable *M. haemolytica* to convert from a commensal to a pathogen, without or with minimal predisposing factors (Griffin et al., 2010).

In contrast to *M. haemolytica*, it is generally assumed that predisposing factors are required for the development of *P. multocida*-associated pneumonia (Griffin et al., 2010). *P. multocida* has 5 capsular serogroups (A, B, D, E and F) and 16 somatic serotypes (1-16), which are associated with, but not completely restricted to, a specific host (in cattle serotype A3 is most prevalent) (Mutters et al., 1986; Griffin et al., 2010;). *P. multocida* have less virulence factors identified compared to *M. haemolytica*. These are a thick

polysaccharide capsula, endotoxin, adhesion proteins (fimbriae, filamentous hemagglutinin, iron acquisition factors), secreted enzymes (neuraminidase) and production of a biofilm, which enable lung infection under predisposing conditions (Ewers et al., 2006). Although *P. multocida* can be highly infectious, the bacteria are not considered to be highly contagious (Booker et al., 1999; Harper et al., 2006). The third member of the Pasteurellaceae family associated with BRD is *Histophilus somni*. *H. somni* are non-encapsulated and produce lipo-oligosaccharides (LOS), various outer membrane proteins (e.g. transferrin binding protein), histamine, a biofilm and immunoglobulin-binding proteins (IgBP's) (Corbeil, 2007; Sandal et al., 2007; Panciera and Confer, 2010). LOS is the primary factor responsible for lesion formation by causing thrombosis, inflammation and tissue destruction (Panciera and Confer, 2010). IgBP's have cytotoxic activities especially towards endothelial cells mediating contraction and allowing hematogenous spread of the bacteria (Corbeil, 2007). As a consequence the clinical presentation of Histophilosis is not limited to pneumonia, but typically includes thrombo-embolic meningo-encephalitis, laryngitis, polyserositis (polyarthritis; pericarditis) and sudden death associated with septicemia-related cardiovascular effects (necrosis of the papillary muscle) (Orr, 1992; Wessels et al., 2004; Wessels and Wessels, 2005).

Several studies have shown that Pasteurellaceae can be detected in the lower airways of both healthy and diseased animals (Autio et al., 2007; Angen et al., 2009). Since most studies used healthy controls housed together with the diseased animals, it cannot be excluded that these controls were subclinically infected, meaning that they could clear the infection without demonstrating clinical signs. As a general concept, Pasteurellaceae are frequently present in the upper airways as part of the normal flora and droplets containing bacteria are constantly inhaled, thereby infecting the lung (Lillie and Thomson, 1972). Whether this lung infection will result in a massive pneumonia associated with clinical signs, depends on the infection pressure and virulence of the specific strain together with preceding damage of viruses and environmental stressors on the one hand and the potency of the calf's innate and specific immunity to clear the infection on the other. Predominantly the bacterial component is responsible for the massive pneumonia and associated production losses (Corbeil and Gogolewski, 1985; Gagea et al., 2006b; Thompson et al., 2006; Schneider et al., 2009). Maternally acquired antibodies against *P. multocida* or *M. haemolytica* in dairy and beef calves began waning

between 2 and 4 weeks of age, whereas autogenous antibody production began at the age of 5 weeks (Hodgins and Shewen, 1998; Prado et al., 2006). This autogenous antibody production occurred in most calves between 60 and 90 days of age, and was not associated with signs of respiratory disease (Prado et al., 2006). This spontaneous seroconversion is likely associated with colonization of the nasal passages with these pathogens as normal flora or with cross-reactive organisms (Prado et al., 2006). Maternal antibodies have been shown to (partly) protect against *Mannheimia haemolytica* infection in lambs (Cowan and McBeath, 1982).

Historically *M. haemolytica* was most frequently isolated and *H. somni* had the lowest prevalence in all production systems. More recent reports indicate that *P. multocida* is nowadays most frequent, possibly due to the widespread use of *M. haemolytica* vaccines (Welsh et al., 2004; Catry et al., 2005; Autio et al., 2007; Nikunen et al., 2007; Angen et al., 2009). In certain systems which commingle calves such as the North American feedlots, the prevalence of *H. somni* and associated disease manifestations is relatively higher (Orr, 1992). Isolation rates (nasal and pulmonary) of all Pasteurellaceae are typically higher in diseased and stressed animals than in apparently healthy animals (Highlander, 2001; Autio et al., 2007; Angen et al., 2009). To date little information on the prevalence of Pasteurellaceae in veal calves is available. In Belgium the nasal prevalence of *P. multocida* in healthy veal calves was markedly higher (33.6%) than for *M. haemolytica* (5.9%) (Catry et al., 2005). In veal calves with and without BRD, *P. multocida* and *M. haemolytica* could be isolated from 1.5% and 4.4% of the broncho-alveolar lavage samples, with isolation rates ranging from 0 to 13.3% of the sampled calves per herd (Arcangioli et al., 2008). Further information on the prevalence in veal calves with BRD is only available from studies evaluating different antimicrobial treatments. In a study evaluating metaphylactic treatment with florfenicol in two Dutch veal farms, the nasal prevalence of *M. haemolytica* and *P. multocida* in diseased calves ranged between 2.3-26.8% and 2.3-9.8%, respectively (Catry et al., 2008). In Swiss veal calves the prevalence of *P. multocida* and *M. haemolytica sensu lato* in transtracheal alveolar lavage samples was 45.0% (18/40) and 42.5% (17/40), respectively (Rérat et al., 2011). *H. somni* was only isolated in 4.9% (2/41) of the cases in a single outbreak in the Netherlands and once (1/40, 2.5%) in an experimental veal set up in Switzerland (Catry et al., 2008; Rérat et al., 2011).

MYCOPLASMATA

Mycoplasmas belong to the class of Mollicutes and are characterized by their tiny physical size and the lack of a cell wall (Maunsell and Donovan, 2009). The latter renders *Mycoplasma spp.* naturally resistant to beta-lactam antimicrobials (Maunsell et al., 2011). Also, they do not synthesize folic acid, leading to natural resistance to sulphonamides (Maunsell et al., 2011). Several species have been identified, of which *Mycoplasma bovis* and *Mycoplasma dispar* are considered pathogenic, whereas *Mycoplasma bovirhinis* is the most frequently isolated commensal (Maunsell and Donovan, 2009). The host pathogen interactions of *M. bovis* are poorly understood. *M. bovis* has a large family of immunodominant variable surface lipoproteins (Vsps), which undergo high frequency phase and size variation, likely contributing to immune evasion and persistence (Beier et al., 1998; Lysnyansky et al., 1999; Buchenau et al., 2010). Next to Vsps, which are adherence molecules, *M. bovis* produces phospholipases, hydrogen peroxide, and superoxide radicals, which damage host cells, and produces a biofilm (Khan et al., 2005; McAuliffe et al., 2006). *M. bovis* can also adhere to neutrophils and inhibit respiratory burst activity (Thomas et al., 1991). Infection with *M. bovis* results in a strong humoral response, with predominantly serum IgG1 and local mucosal IgG and IgA responses (Howard et al., 1980; Howard and Gourlay, 1983; Vanden Bush and Rosenbusch, 2003). IgG concentrations in broncho-alveolar fluid have been associated with resistance to *M. bovis* associated respiratory disease, suggesting protection by maternal immunity (Howard et al., 1980). However, no association between *M. bovis* serum antibody titers in the first weeks of life and occurrence of pneumonia or *M. bovis* associated disease could be demonstrated (Van Donkersgoed et al., 1993; Brown et al., 1998).

M. bovis has been associated with mastitis, pneumonia, otitis media (via the Eustachian tube), arthritis (through septic spread), synovitis, periarticular infection, keratoconjunctivitis, meningitis (through otitis media or via septic spread), cardiac disease (myo- and endocarditis) and even genital disorders (abortion, vesiculitis) (Maunsell and Donovan, 2009; Maunsell et al., 2011). When occurring in association with BRD, arthritis and otitis are collectively termed *M. bovis* associated disease (MbAD) (Maunsell and Donovan, 2009). The occurrence of *M. bovis* induced otitis media appears to be age related with peak incidences at 2 to 6 weeks of age (Walz et al., 1997). Also for

M. bovis induced pneumonia, the peak incidence is reached around a month of age in endemic dairy herds (Bennett and Jasper, 1977; Brown et al., 1998). The exact contribution of *M. bovis* to the BRD complex is not as easily understood. Although *M. bovis* can experimentally induce pneumonia, especially in young calves (3-12 weeks), the presence of *M. bovis* in the upper and lower respiratory tract does not necessarily result in clinical disease under field conditions (Nicholas et al., 2002; Vanden Bush et al., 2003). Under experimental conditions, infection with *M. bovis* alone in calves at feedlot age (> 6 months) resulted only in a short-lasting increase in temperature (<39.5°C) and very limited pulmonary changes at necropsy 18 days later (Prysliaik et al., 2011). Mainly based on necropsy studies in feedlot cattle, it appears that *M. bovis* can substantially contribute to morbidity and mortality (Shahriar et al., 2002). Especially chronic, unresponsive BRD has been associated with *M. bovis* pneumonia, often in combination with detection of BVDV virus (Shahriar et al., 2002). Co-infection of *M. bovis* with *M. haemolytica*, *H. somni* and *P. multocida* is extremely common in infected herds (Stipkovits et al., 2000; Gagea et al., 2006a; Fulton et al., 2009).

Introduction of an asymptomatic carrier is believed to be the primary cause of introducing *M. bovis* in free herds (Jasper, 1981; Tschopp et al., 2001). Calves can remain infected for long periods of time and may shed *M. bovis* intermittently for many months to years, acting as reservoirs in the herd (Bennett and Jasper, 1977; Pfutzner and Sachse, 1996). Despite the thin cell membrane, *M. bovis* can survive up to 2 months in milk or sponges at 4°C, making the environment a potential source of infection (Pfutzner and Sachse, 1996). However, survival dropped considerably with higher environmental temperatures (Pfutzner and Sachse, 1996). Next to drinking infected milk in dairy calves, few specific risk factors for *M. bovis* infection have been identified (Maunsell and Donovan, 2009). Mixing of calves from different sources and the presence of at least one seropositive calf in new purchases increased the risk of MbAD (Tschopp et al., 2001). An interesting feature for veal production, is the fact that male mice are more susceptible for mycoplasmal infection, suggesting a hormonal influence on disease susceptibility (Yancey et al., 2001).

Worldwide emergence of *M. bovis* is reported, predominantly in large scale production (e.g. North American dairy industry) or in systems that commingle calves (Van Donkersgoed et al., 1993; Kirk et al., 1997; Arcangioli et al., 2008; Assie et al., 2009b). *M.*

M. bovis is present in most European countries, but for example could not be detected in BRD outbreaks in Finland (Härtel et al., 2004; Nikunen et al., 2007). The presence of *M. bovis* in the Dutch veal industry has been reported as early as in 1992 (ter Laak et al., 1992). In a recent French study on vaccinated veal calves on straw, 89% (8/9) of the herds were positive with a within herd prevalence of 89% (range: 67-100%) (Arcangioli et al., 2008). Also, in Italian veal calves at slaughter, 100% of veal calves were seropositive and *M. bovis* could be isolated from 25% (16/64) of calves with pneumonic lesions at that time (Radaelli et al., 2008). Coinfection of *M. bovis* with *Pasteurella spp.* has been found in 18.6% of the calves with pneumonia at slaughter (Soehnlén et al., 2012). In broncho-alveolar samples of 10 diseased and 5 healthy animals per cohort, coinfection of *M. bovis* and Pasteurellaceae involved 63.7% of the culture positive samples (Arcangioli et al., 2008). Nasal and pulmonary colonization of *M. bovis* in veal calves, started immediately after arrival and the highest prevalence was reached 50 days after arrival (Soehnlén et al., 2012). The prevalence of *M. bovis* in veal calves in Belgium is currently unknown, as are its importance in the BRDC of veal calves and its relation with other BRD pathogens.

ENVIRONMENTAL FACTORS

Next to calf factors and infectious agents, environmental factors such as housing, grouping or transport play an important role in the BRDC (Arthington et al., 2003; Svensson and Liberg, 2006; Svensson et al., 2006a; Lomborg et al., 2008; Step et al., 2008; Bach et al., 2011). The current marketing system of veal calves in Europe inherently causes stress to the animals, resulting in a higher risk for BRD. First, commingling with calves from multiple origin with unknown disease and immunity (maternal immunity and vaccination) status occurs at a sorting center (= sale barn). Commingling and passage through a sale barn have both been documented as major BRD risk factors in feedlots and dairy calves (Gummow and Mapham, 2000; O'Connor et al., 2005; Sanderson et al., 2008; Step et al., 2008; Bach et al., 2011). Also transport, especially the length of transport, has been associated with an increased BRD and mortality risk (Staples and Haugse, 1974; Cave et al., 2005; Sanderson et al., 2008). Veal calves are transported twice, first from the herd of origin to the sorting center and later from the sorting center to the veal herds. With exception of the import of eastern European or British calves, transportation times in veal calves are much shorter than in North American feedlots. The veal industry complies with the European legislation on transport of animals and has made extra efforts to assure animal welfare during transport, for example through the use of conditioned trucks. Nevertheless, transport potentially exposes calves to additional commingling, chilling, dehydration, exhaustion and starvation causing stress and immunosuppression (Blecha and Minocha, 1983; Blecha et al., 1984; Buckham Sporer et al., 2007; Buckham Sporer et al., 2008; Sporer et al., 2008).

At arrival at the veal herd additional stressors include extra commingling with calves from earlier transports, dietary changes, adaptation to drinking from a bucket and different housing conditions. High pressure cleaning immediately prior or at arrival of a new batch of calves may leave infective aerosols and increase the BRD risk (Palechek et al., 1987). Veal calves are predominantly housed on slatted floors, which have been associated with a higher BRD risk than straw boxes in an observational study in French beef calves, possibly because of higher ammonia levels (Assie et al., 2004). The BRD risk also increases with increasing number of animals present in a stable (Assie et al., 2004; Gay and Barnouin, 2009; Gulliksen et al., 2009). Therefore, the large number of calves

per veal herd poses an additional risk for BRD. In dairy calves, age differences larger than 8 weeks between groups of calves have been associated with a higher disease risk in the younger groups (Gulliksen et al., 2009). Veal calves within a cohort show relatively limited age differences, but when two different cohorts are present on one farm, large age differences between both cohorts might exist, putting the younger calves at an increased risk. Repeated regrouping in the first weeks after arrival is necessary to ensure homogenous growth of the calves, but this leads again to additional commingling and potentially BRD. Season plays an important role, and calves are at a higher risk for BRD in autumn and winter in all production systems (Andrews, 1976; Loneragan et al., 2001; Svensson et al., 2006a). This seasonal effect is predominantly caused by weather conditions (e.g. fog) (Ribble et al., 1995; Assie et al., 2004). It is important to note that the seasonal effect might be biased by an increased density of cattle in a certain season associated with calving (Ribble et al., 1995). Also in veal calves a higher incidence of BRD in winter and fall has been reported (R. Boone, Personal communication). The only advantage that veal calves might have, compared to dairy or beef calves, is that they are not weaned, which is a major stress factor in other production systems (Lomborg et al., 2008).

The relationship between nutrition, immunity and BRD has extensively been studied in different production systems, but not in veal calves. Recent reviews concluded that no definitive link between BRD and most specific nutritional factors could be established (Galyean et al., 1999; Duff and Galyean, 2007; Taylor et al., 2010a). Instead of the ration composition, achieving adequate feed intake might be more important to combat BRD and its consequences. The APR, but also chronic inflammation, have increased protein and caloric costs, and therefore adequate feed intake is essential (Corbeil and Gogolewski, 1985; Blum et al., 1996; Barnes et al., 2002). Also, calves require more nutrition to achieve equal growth in a cold environment (Nonnecke et al., 2009). Part of the reduction in growth is attributed to the reduced feed intake, which is observed in calves suffering from BRD (Sowell et al., 1999; Buhman et al., 2000; Cusack et al., 2007). More important, stress, which is abundantly present in veal calves in the first weeks after arrival, reduces feed intake making animals more susceptible for the consequences of infection (Loerch and Fluharty, 1999). The diet of veal calves is highly specific, and little is known on its relationship with disease. One study showed that additional provision of solid feed (wheat straw or dried beet pulp) decreased the number of

treatments for BRD (Cozzi et al., 2002). Next to general nutritional state, deficiencies in several trace elements (Fe, Copper, Zinc, Iodine and Selenium (Se)) are associated with impaired immune function (Guyot et al., 2009). The consequences of the Fe deficient state in which veal calves are maintained have been discussed in the previous chapter. Se deficiency has several consequences, including an impaired innate immunity. For example the level of circulating mononuclear cells decreased severely in Se-deficient cattle (Sordillo et al., 1993). Se-deficient calves were still able to mount an immune response after challenge (BHV-1 or *M. haemolytica*) in several studies, but to a lesser extent (lower antibody levels) than calves with adequate Se levels (Reffett et al., 1988; Stabel et al., 1989). Since Se deficiencies are common in Belgian calves, especially in beef calves, this potentially increases the BRD risk in the white veal industry (Guyot et al., 2009).

CONCLUSION

Based on existing knowledge from other production systems, it can be stated that veal calves are at ultra-high risk of developing BRD under the present organization of the industry. The present management is unable to overcome disease, making antimicrobial therapy an absolute necessity to guarantee acceptable production results and animal welfare. Whereas the influence of certain calf and environmental factors can be well extrapolated from other production systems, the prevalence and relative importance of the known BRD pathogens in the veal calf BRDC remains to be determined.

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CHAPTER 2

SCIENTIFIC AIMS

The emergence of antimicrobial resistance in commensal, pathogenic and zoonotic bacteria from veal calves is worrisome, potentially threatening treatment options in both human and veterinary medicine. For economic reasons and due to a lack of epidemiological knowledge on diseases in veal calves, veterinarians have been tied to empiric or blind treatment in the veal industry for decades. In line with this problematic situation, the overall objective of this doctoral thesis was to gain insights into current practices, into the epidemiology of morbidity and mortality in white veal calves and into the underlying pathogens. This to provide the industry with objective data from where sustainable preventive and therapeutic protocols can be installed and evaluated in the future.

The specific objectives of the present thesis were:

- (1) To describe quantitative and qualitative drug use and associated risk factors in white veal calves in Belgium (Chapter 3)
- (2) To determine causes and timing of mortality and morbidity in white veal production systems in Belgium (Chapter 4.1)
- (3) To determine the impact of respiratory disease, diarrhea, arthritis and otitis on mortality and carcass traits in white veal calves (Chapter 4.2)

Because bovine respiratory disease (BRD) was the leading cause of mortality, morbidity and drug use, additional objectives were:

- (4) To determine the prevalence of respiratory pathogens in white veal calves with respiratory disease (Chapter 5.1)
- (5) To determine the seroepidemiology of respiratory infections in white veal calves and their association with BRD and carcass traits (Chapter 5.2)
- (6) To evaluate the value of total immunoglobulin concentration and the serostatus for respiratory pathogens, measured at arrival, for the prediction of BRD and carcass traits in white veal calves (Chapter 5.2)

CHAPTER 3
DRUG USE
IN WHITE VEAL CALVES

PROSPECTIVE STUDY ON QUANTITATIVE AND QUALITATIVE ANTIMICROBIAL AND ANTI- INFLAMMATORY DRUG USE IN WHITE VEAL CALVES

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ABSTRACT

Objectives: To document and quantify drug use in white veal calves, an intensive livestock production system where multidrug resistance is abundantly present.

Methods: Drug consumption data were prospectively collected on 15 white veal production cohorts (n= 5853 calves) in Belgium (2007–09). Treatment incidences (TIs) based on animal defined daily dose (ADD), prescribed daily dose (PDD) and used daily dose (UDD) were calculated. Risk factors were identified by linear regression.

Results: The average TI_{ADD} of antimicrobial treatments was 416.8 ADD per 1000 animals at risk. Predominantly, oral group antimicrobial treatments were used (95.8%). Of the oral group antimicrobial treatments, 12% and 88% were used for prophylactic or metaphylactic indications, respectively. The main indication for group and individual drug use was respiratory disease. The most frequently used antimicrobials (group treatments) were oxytetracycline (23.7%), amoxicillin (18.5%), tylosin (17.2%) and colistin (15.2%). Deviations from the leaflet dosage recommendations were frequently encountered, with 43.7% of the group treatments underdosed (often oxytetracycline and tylosin to treat dysbacteriosis). In 33.3% of the oral antimicrobial group treatments a combination of two antimicrobial preparations was used. Smaller integrations used more antimicrobials in group treatments than larger ones ($P<0.05$); an integration is defined as a company that combines all steps of the production chain by having its own feed plant and slaughterhouse and by placing its calves in veal herds owned by producers that fatten these calves for this integration on contract. Producers used higher dosages than prescribed by the veterinarian in cohorts with a single caretaker ($P<0.01$).

Conclusions: The present study provided detailed information on the intensive antimicrobial use in the white veal industry. Reduction can only be achieved by reducing the number of oral group treatments.

INTRODUCTION

Antimicrobial resistance is one of the leading health concerns in human and veterinary medicine worldwide (Hawkey and Jones, 2009). Within the different animal husbandry systems, the highest levels of antimicrobial resistance are found in pigs, poultry and veal calves (Catry et al., 2005; Catry et al., 2006; Catry et al., 2007a Di Labio et al., 2007; Hendriksen et al., 2008a,b; Persoons et al., 2010). These intensively reared livestock production animals receive multiple antimicrobial group medications (Timmerman et al., 2006; Persoons, 2011). Transfer to humans, eventually leading to therapy failure, might occur through direct contact with live animals or indirectly via contaminated meat or the environment (Philips et al., 2004; Vanderhaeghen et al., 2010; Johnson, 2011). A clear association between antimicrobial drug use and the appearance of antimicrobial resistance has been demonstrated under different conditions (Tenover and McGowan, 1996; Berge et al., 2006; Donaldson et al., 2006; Jensen et al., 2006; Checkley et al., 2010). Also underdosing has been documented as a risk factor for the development of antimicrobial resistance (David and Gill, 2008). Because of the great variation between countries, production systems and producers, collection of standardized data is recommended for timely and regional comparisons (WHO, 2002; Timmerman et al., 2006; Gonzalez et al., 2010; Persoons, 2011). Such comparisons are also required for a proper evaluation of interventions. These studies have been published for pigs, poultry and conventional cattle, but not for veal calves (Grave et al., 1999, 2004; Timmerman et al., 2006; Pol and Ruegg, 2007; Gow and Waldner, 2009; Gonzalez et al., 2010; Persoons, 2011; Vieira et al., 2011).

Knowledge on antimicrobial consumption in veal calves is of particular interest, since the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA ST398) at the herd level is by far the highest (88%) among European livestock production systems and 33% of the veal producers are MRSA positive (Graveland et al., 2010). Also, there is a growing interest in the use of (non-)steroidal anti-inflammatory drugs ((N)SAID's) as an additional or replacement therapy for antimicrobials in veal calves. Despite this evolution, there are hardly any studies documenting and quantifying the current use of non-antimicrobial drugs in veterinary medicine (Smith et al., 2008).

Therefore the objectives of the present study were as follows: (1) to describe and quantify group and individual antimicrobial and anti-inflammatory drug use, (2) to determine risk factors for antimicrobial use and deviation from norm-dosing (leaflet recommendations) in group treatments at the cohort level.

METHODS

STUDY DESIGN

The study population consisted of veal calf herds located in Flanders (Northern Belgium), which were in compliance with the Belgian Controlled Veal (BCV) label. The sampling frame was the list of veal herds in Flanders officially registered in the Belgian cattle registration system (SANITEL, administered by the Animal Health Service-Flanders). Of the 295 veal herds in Belgium, 285 herds (97%) are situated in Flanders and 271 herds (95%) complied with the BCV label. Because of the intensive registration, the regular visits and the continuous reporting required in the fulfillment of this survey, farms were conveniently selected to assure optimal collaboration of the producers. Selection criteria included the willingness to keep detailed registration records on diseases and treatments and allowing the use of all farm data, available in the Belgian registration system (SANITEL). Selection was independent of any disease history and for logistic reasons the farms were gradually initiated in the study between October 2007 and October 2009. The study group consisted out of 15 production cohorts, following 15 herds, which were stratified on breed (5 production cohorts per breed: Holstein Friesian (HF), Belgian Blue (BB), and crossbreeds (HFxBB), respectively). A production cohort was defined as one all in all out production cycle. One herd can have different production cohorts at the same time, but here only one production cohort per herd was included as the primary epidemiological unit of interest. The study period included the complete production cycle from arrival to slaughter. Veal production in Europe is typically integrated. An integration is defined as a company which combines all steps of the production chain by owning its own nutrition plant and slaughter house and by placing its calves in veal herds owned by producers which fatten these calves for this integration on contract. Calves belonging to the same production cohort were housed in the same stable. All calves were individually housed during the first 6 weeks after which

they were housed in groups of on average 6 animals (range: 2-8), on slatted floors in compliance with the Belgian legislation. None of the calves were vaccinated.

DATA COLLECTION

Herds were visited at calf arrival and provided with preprinted treatment recording forms. All individually administered treatments (both oral and parenteral) were daily recorded on these forms by the producer. Recording of the treatments consisted of calf identification (ear tag), drug name, dose (mL) and administration route. The following treatment indications were optioned on the registration form: respiratory disease, diarrhea, (idiopathic) peritonitis, acute ruminal disorder, ruminal drinking, otitis, arthritis, omphalitis, laryngeal necrobacillosis, nervous symptoms, and miscellaneous. All treatments, individually administered by the veterinarian, were recorded on the treatment forms as well. Not included as treatments were the administration of iron (to control the anemic state of the calves), electrolytes, pectins or probiotics. For group treatments the indication, the drug name, the dose (mg/kg body weight) and the prescribed therapy duration (length in days) were registered on a separate form by the farmer. A group treatment was defined as each prophylactic or metaphylactic administration of a drug to a minimum of one complete compartment. Prophylactic use was defined as each treatment of healthy animals to prevent disease from occurring. Metaphylactic use was defined as the simultaneous treatment in a shared compartment of clinically healthy animals and animals that showed clinical symptoms of the disease (Aarestrup, 2005). After slaughter, the collected written treatment records were compared with the mandatory prescription documents (administration records of the veterinarian and the official medication register of the farmer), which are under supervision of the federal agency for safety of the food chain. Herds were visited between 4 and 8 times by the same investigator to check compliance with the registration system.

PROCESSING OF ANTIMICROBIAL AND ANTI-INFLAMMATORY CONSUMPTION RECORDS

Group and individual treatment data were entered in a relational database (Access 2010, Microsoft inc., Redmond, WA, USA). Volumes of antimicrobials and anti-inflammatory drugs administered were converted to mg of active substance per kg live body weight. Drug consumption records were processed using three units of measurements outlined

in detail below: the animal defined daily dose (ADD), the prescribed daily dose (PDD) and the used daily dose (UDD) (Grave et al., 1999; Chauvin et al., 2001; Grave et al., 2004; Jensen et al., 2004, 2006; Timmerman et al., 2006; Pol and Ruegg, 2007; Gonzalez et al., 2010; Persoons, 2011; Vieira et al., 2011).

ADD is defined as the average maintenance dose for the main indication in a specified species, for example 30 mg/kg oxytetracycline for bovine respiratory disease (Jensen et al., 2004). ADD values (mg drug per kg live weight) were estimated for each antimicrobial and anti-inflammatory substance, based on the dosage recommendations of the Belgian compendia for veterinary drugs and the drug instructions leaflets (BCFI, 2009). The Anatomical Therapeutic Chemical classification system for Veterinary medicinal products (ATCVet) was used for antimicrobial drug identification (WHO, 2002). For the combination preparations the ADD were estimated for the main substance with the exception of trimethoprim/sulphonamide combinations, for which the ADD was set for both molecules (Dumartin et al., 2010). For long-acting preparations the ADD was calculated from the recommended dosage into a 24 hours dose, by dividing by a long acting factor 2 for amoxicillin, danofloxacin, florfenicol and tilmicosin, and 5 for tulathromycin.

The PDD reflects the prescribing behaviour of the veterinarian, and was calculated by dividing the dosage mentioned on the official drug prescription records, delivered by the veterinarian, by the average live weight at the beginning of the treatment. For example for bovine respiratory disease, a PDD can be 2 gram oxytetracycline per day per calf, irrespective of the exact body weight. The UDD is calculated as the amount of an antimicrobial drug administered during a given period (days) divided by the number of calves at risk and their average live weight at the beginning of the treatment (Timmerman et al., 2006). In this way the UDD reflects the dose, truly administered by the producer, for example 18 mg/kg body weight oxytetracycline for bovine respiratory disease in a particular herd. The kg of animals at risk was determined by multiplying the number of animals present at the beginning of a given treatment with the estimated average body weight at that time (Jensen et al., 2004). Average live weight curves per week after arrival and per breed (HF, BB and HFxBB) were created, based upon the feed uptake and slaughter data of the monitored herds. Standard values for average weight at arrival (42 kg for HF, 46 kg for BBxHF and 56 kg for BB), feed conversions (0.46 kg

growth/kg milk powder for HF, 0.64 for HFxBB and 0.76 for BB) and killing out percentages (55% for HF, 63% for HFxBB and 69% for BB) were obtained from the veal integrators.

The frequency of treatment was quantified by calculating the treatment incidence (TI), based upon the 3 definitions of defined dose (DD) explained above, namely ADD (TI_{ADD}), PDD (TI_{PDD}) and UDD (TI_{UDD}) (Grave et al., 1999; Timmerman et al., 2006; Persoons, 2011). In order to be able to compare with the monitoring of antimicrobial usage in animals in the Netherlands (MARAN), the treatment incidences based on ADD were also calculated for the standard veal calf live weight of 164 kg, as used in that report (TI_{ADDsw}) (MARAN-2009, 2011).

The following formula was used to calculate treatment incidences:

$$TI_{ADD} \text{ or } TI_{PDD} \text{ or } TI_{UDD} = \frac{\text{Total amount of drug administered (mg)}}{ADD, PDD \text{ or } UDD \left(\frac{\text{mg}}{\text{kg}}\right) * \text{number of days at risk} * \text{kg veal}} \times 1000$$

The TI for veal calves is defined as the number of calves per 1000 that is treated daily with one ADD, PDD or UDD, respectively. In order to estimate the TI's as precise as possible, these were calculated per installed treatment, using the average weight at the time of treatment. Overall treatment indices were then calculated as the sum of all TI's in a cohort. The relative importance of each administered antimicrobial was expressed by the proportional TI_{ADD}, TI_{PDD} and TI_{UDD}. These were calculated by dividing the TI_{ADD}, TI_{PDD} or TI_{UDD} of each antimicrobial by the total TI_{ADD}, TI_{PDD} or TI_{UDD}, respectively (Timmerman et al., 2006).

Both for antimicrobials and (N)SAID's the PDD/ADD and UDD/ADD ratios were calculated to assess the compliance with dosing by the veterinarian or the farmer, respectively. A ratio lower and higher than 1, respectively, was considered as under- and overdosing, taking an acceptable inaccuracy of 0.2 into account (Timmerman et al., 2006). In the same way the UDD/PDD ratio reflects to what extent the farmer actually applied the doses prescribed by the veterinarian. For the oral group treatments TI's and the 3 different dosing ratios were calculated for every installed treatment, whereas for individual treatments these were calculated per drug and per indication on a weekly basis. All results are displayed as mean (standard deviation (SD); median; minimum-maximum).

STATISTICAL ANALYSIS

Cohort level predictors (n= 18) were collected through a personal interview with the producers or derived from the technical results of the cohort. These included breed (dairy, mixed breed or beef calves), production cycle length (> 196 days; < 196 days), herd location (province), region (West or East of Flanders), herd size (<600 calves; 600-900; >900), number of cohorts per herd (1/ more than 1), cohort size (<300 calves; 300-500; >500), year of arrival (2007, 2008, 2009), season of arrival, compartmentalization (1 compartment/ >1), mortality risk of the studied cohort (low: <3%; intermediate: 3-6 and high: >6%), mortality risk due to pneumonia of the studied cohort (low: <1%; intermediate: 1-2%; high: >2%), identity of the veterinarian, identity of the integrator, integration size (≥ 50 herds; <50 herds), number of caretakers (1/ more than 1), gender of the primary caretaker (male/female), presence of foreign calves (yes/no) and presence of other food animals (yes/no). An origin index was calculated by dividing the number of herds of origin by the number of calves that arrived at the cohort (<0.7= few herds of origin; 0.7-0.8= moderate; > 0.8= high) and added as a cohort level risk factor.

Risk factors for group antimicrobial use and correctness of dosing at the cohort level were identified using linear regression models with respectively the total TI_{UDD} for group antimicrobial treatments and the average of the UDD/ADD ratio as continuous outcome variables. In a first step all predictors were tested univariable. The variables with a *P*-value of 0.2 or less were withheld for the multivariable regression model. Pearson and Spearman's rho correlation coefficients were calculated, and if correlation between two selected predictors was higher than 0.6, only the most significant variable was retained in the model. When the log likelihood changed substantially after removal of a non-significant predictor, the predictor was retained in the model. The model was built stepwise backwards, gradually excluding the non-significant factors and finally only retaining the significant factors. Significance was set at $P \leq 0.05$. Interactions were checked for all significant main factors in the model. All models were built in S-plus 8.2 (Tibco Spotfire, Somerville, Massachusetts, USA).

RESULTS

QUANTITATIVE AND QUALITATIVE DRUG USE

Drug use was monitored in 5853 veal calves, housed in 15 commercial veal herds, with an average herd size of 679 (SD=334) calves (964 (SD=418) for dairy, 588 (SD=112) for crossbreeds and 484 (SD=207) for beef calves). Herd sizes of the selected herds were comparable to the sampling frame (Student's t-test, $P>0.05$). The mean production length was 196 days (range: 175 to 211). The individual treatment records were judged as unreliable after comparison with official treatment registration records in 5 cohorts. Therefore, individual drug use could only be processed for 3519 calves (10 production cohorts).

Group treatments were by far more frequently used than individual treatments (97.9% of the total use, based on UDD). All antimicrobial group treatments were orally administered, twice daily in the milk. The average number of group treatments courses per production cohort was 10 ± 3 (range: 4-15). Antimicrobials accounted for 82.0% (mean: 8; SD: 3; range: 4-13) of the group treatments and non-antimicrobials (macrocyclic lactones and (N)SAID's) for 18.0% (mean: 2; SD:1; range: 1-4). Of the antimicrobial group treatments ($n=126$), 13.0% was used prophylactic (immediately after arrival) and 87.0% metaphylactic or curative. Most group treatments were administered in the first weeks after arrival (Figure 1). The most frequent indication for antimicrobial group treatment was respiratory disease (53%). Other indications were arrival prophylaxis (13%), diarrhea (12%), dysbacteriosis (defined as non-specific bacterial enteritis) (12%), idiopathic peritonitis (7%) and enterotoxaemia (3%). In 33.3% of the antimicrobial group treatments a combination of two antimicrobial preparations was used. The applied antimicrobial combinations by indication are given in Table 1. The average (standard deviation; median; min.- max.) TI_{ADD} for all antimicrobial group treatments was 414.0 (149.6; 420.4; 111.5-816.7), meaning that on average per day 414 veal calves out of 1000 were treated with one ADD. However, in reality less calves were treated, namely 379 ($TI_{UDD}= 379.0$ (121.3; 346.1; 111.2-656.6)), indicating an overall overdosing. When comparing the average TI_{UDD} with the average TI_{PDD} ($=387.0$ (120.5; 373.7; 158.7-585.1)), the difference is small, indicating that, for the group treatments, producers on average followed the prescriptions of the veterinarians.

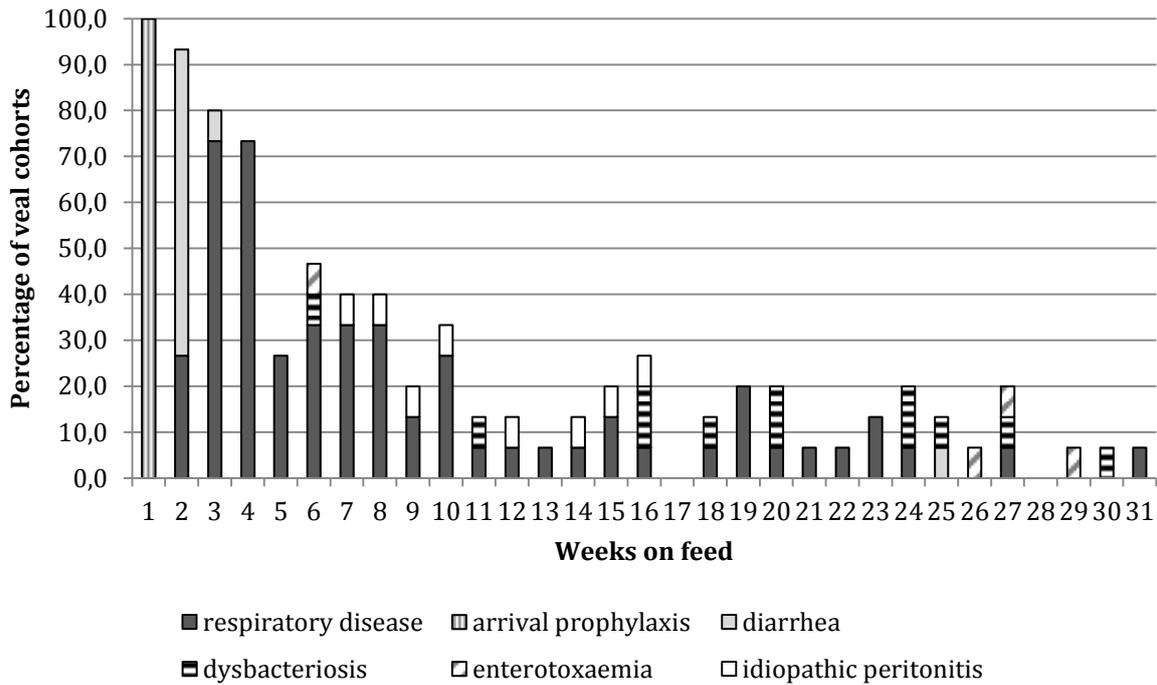


Figure 1. Percentage of veal cohorts (n=15) receiving antimicrobial group treatment at least four days of the week by indication and by week of production (2007-2009, Belgium).

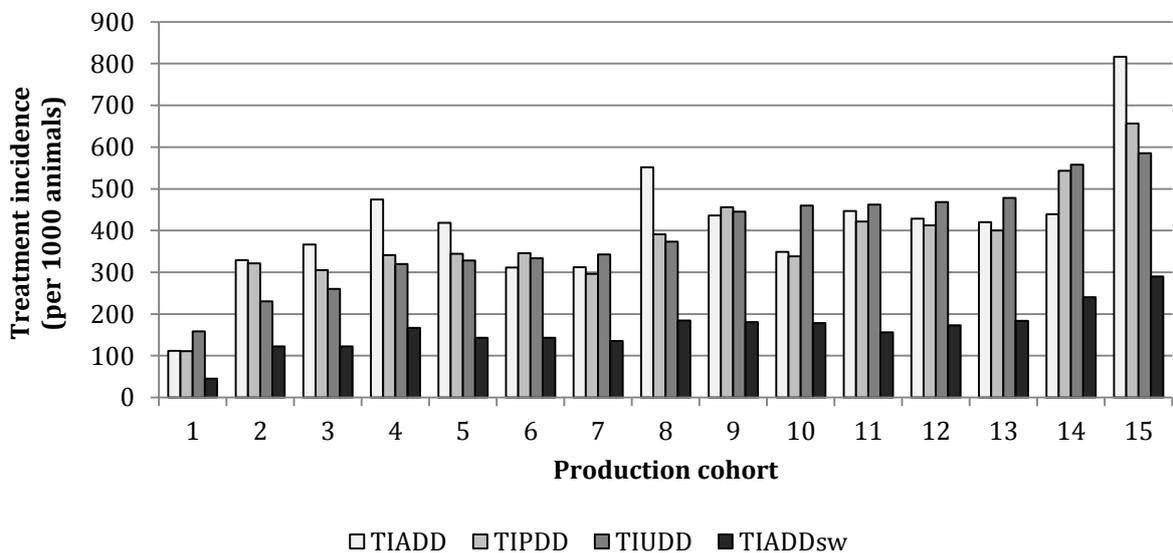


Figure 2. Comparison of the treatment incidence based on defined daily dose (TI_{ADD}), defined daily dose for a standard weight of 164 kg (TI_{ADDsw}), prescribed daily dose (TI_{PDD}) and used daily dose (TI_{UDD}) for group treatments on 15 white veal production cohorts, ranked by increasing TI_{UDD} (15 production cohorts, 5853 calves, 2007-2009, Belgium).

Table 1. Use and combination of oral antimicrobial group treatments in white veal calves by indication, 2007-2009 (15 cohorts, 5853 calves, Belgium)

Active substance	Indication (number of group treatments (% within the indication))						Total
	Arrival prophylaxis	Respiratory disease	Diarrhea	Dysbacteriosis	Idiopathic peritonitis	Enterotoxaemia	
Amoxycillin		3 (4.5)			4 (44.4)	1 (25.0)	8 (6.3)
Ampicillin					1 (11.1)		1 (0.8)
Tylosin		5 (7.5)		3 (20.0)		3 (75.0)	11 (8.7)
Tilmicosin		1 (1.5)					1 (0.8)
Trimethoprim + sulphonamides		4 (6.0)					4 (3.2)
Oxytetracycline		16 (23.9)		9 (60.0)	1 (11.1)		26 (20.6)
Doxycycline		16 (23.9)					16 (12.7)
Flumequine			14 (93.3)				14 (11.1)
Enrofloxacin			1 (6.7)				1 (0.8)
Colistin		1 (1.5)			1 (11.1)		2 (1.6)
Amoxycillin + flumequine					2 (22.2)		2 (1.6)
Amoxicillin + tylosin		3 (4.5)					3 (2.4)
Amoxicillin + colistin	13 (81.3)						13 (10.3)
Tylosin + oxytetracycline		9 (13.4)		3 (20.0)			12 (9.5)
Tylosin + doxycycline		9 (13.4)					9 (7.1)
Tylosin + trimethoprim + sulphonamides	1 (6.3)						1 (0.8)
Trimethoprim + sulphonamides + colistin	2 (12.5)						2 (1.6)
Total (% of overall total)	16 (12.7)	67 (53.2)	15 (11.9)	15 (11.9)	9 (7.1)	4 (3.2)	126

The TI_{ADDsw} , based on the standard weight of 164 kg as applied in the Dutch MARAN report, was much lower, namely 164.3 calves per 1000 (55.0; 166.9; 45.1-289.7) (Figures 2 and 3). The most frequently used antimicrobials for group treatments were oxytetracycline (proportional TI_{UDD} = 23.7%), amoxicillin (proportional TI_{UDD} = 18.5%), tylosin (proportional TI_{UDD} = 17.2%) and colistin (proportional TI_{UDD} = 15.2%), which were used on 93.3% of the production cohorts (Figure 3, Tables 2 and 3). At the cohort level, on average 43.7% (17.9; 42.9; 11.1-69.2) of the oral antimicrobial group treatments was underdosed (UDD/ADD ratio <0.8) and 37.1% (12.9; 35.7; 18.8-61.5) was overdosed (UDD/ADD ratio > 1.2) (Table 2). The main reason for underdosing was the use of oxytetracycline and tylosin for the treatment of dysbacteriosis, which was most frequently treated in the second half of the production cycle (Table 4 and Figure 1).

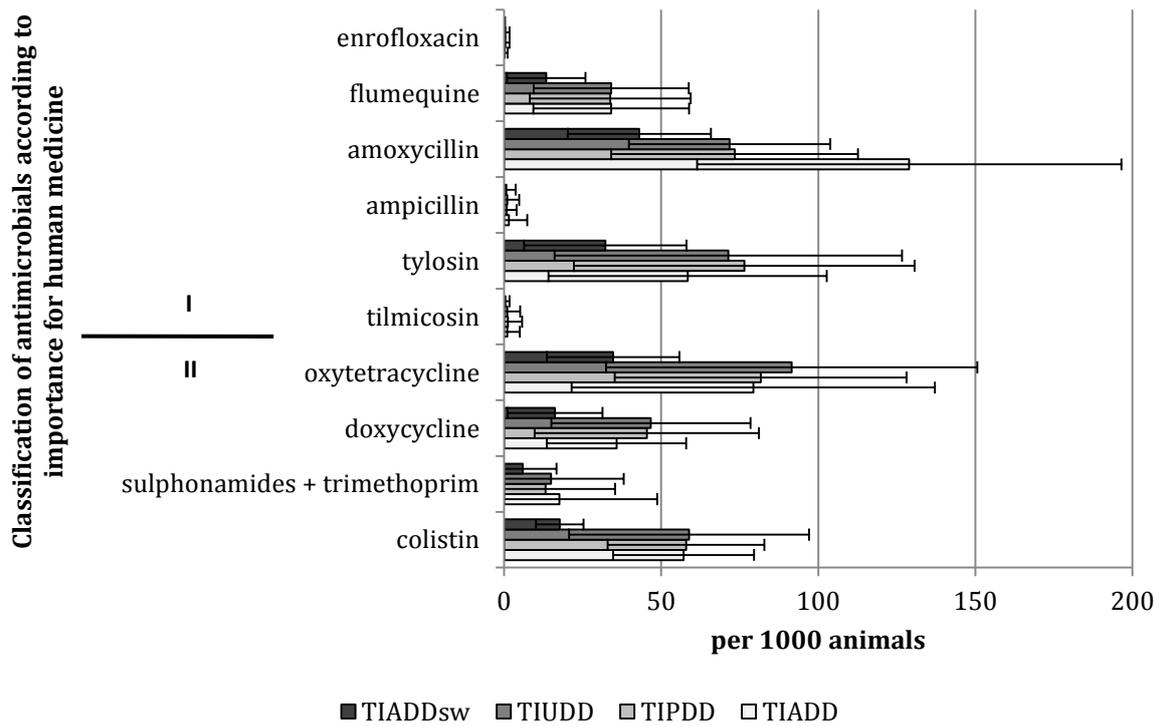


Figure 3. Treatment incidence (number of animals per 1000 treated daily with one dose) based on animal defined daily dose (TI_{ADD}), prescribed daily dose (TI_{PDD}), used daily dose (TI_{UDD}), and defined daily dose for a standard live weight of 164 kg (TI_{ADDsw}) of group treatments in white veal calves per registered antimicrobial compound, classified according to their importance in human medicine (WHO, 2007). Class I: critically important; class II: highly important antimicrobials for human medicine. Error bars represent the standard deviation (15 cohorts, 5853 calves, 2007-2009, Belgium).

Table 2. Daily dosages (mg/kg) and dosing ratios of oral antimicrobial group treatments in white veal calves, 2007-2009 (15 cohorts, 5853 calves, Belgium)

Active substance	ATCvet ^a	ADD	PDD	UDD	PDD/ADD ratio	UDD/ADD ratio	UDD/PDD ratio	Total use ^b	Frequency of use
Mean±SD (min-max)								kg (%)	(% of cohorts)
Amoxicillin	QJ01CA	15	25.7±5.2 (18.9-34.8)	26.4±7.2 (15.2-36)	1.7±0.4 (1.3-2.3)	1.8±0.5 (1.0-2.4)	1.0±0.3 (0.6-1.7)	109.3 (23.8)	93.3
Ampicillin	QJ01CA	20	37.0	30.8	1.9	1.5	0.8	6 (1.3)	6.7
Tylosin	QJ01FA	16	15.4±7.2 (6.5-36.4)	15.5±6.7 (7-29.9)	1.0±0.5 (0.4-2.3)	1.0±0.4 (0.4-1.9)	1.1±0.6 (0.5-2.8)	73.4 (15.9)	93.3
Tilmicosin	QJ01FA	20	17.2	19.3	0.9	1.0	1.1	1.44 (0.3)	6.7
Trimethoprim-Sulfonamides	QJ01EW	30	37.4±10.9 (21.4-48.3)	35.9±21.0 (7.1-55.9)	1.3±0.4 (0.7-1.6)	1.2±0.7 (0.2-1.9)	1.0±0.6 (0.3-1.7)	8.0 (1.7)	33.3
Oxytetracycline	QJ01AA	30	28.6±12.4 (9.3-46.3)	30.1±20.9 (4.8-75.8)	1.0±0.4 (0.3-1.5)	1.0±0.7 (0.2-2.5)	1.0±0.4 (0.4-2.2)	185.8 (40.4)	93.3
Doxycycline	QJ01AA	10	8.9±2.1 (6.5-14.3)	8.2±3.9 (3.7-18.3)	0.9±0.2 (0.7-1.4)	0.8±0.4 (0.4-1.8)	1.0±0.5 (0.5-2.3)	28.8 (6.2)	100
Flumequine	QJ01XB	12	12.3±2.4 (8.6-15.9)	12.6±4.0 (7.2-19.1)	1.0±0.2 (0.7-1.3)	1.1±0.3 (0.6-1.6)	1.0±0.3 (0.6-1.5)	31.7 (6.9)	86.7
Enrofloxacin	QJ01MA	3.75	2.5	2.5	0.7	0.7	1.0	0.04 (<0.01)	6.7
Colistin	QJ01XB	5	5.0±0.5 (4.2-5.5)	5.4±1.5 (2.8-8.2)	1.0±0.1 (0.8-1.1)	1.1±0.3 (0.6-1.6)	1.1±0.3 (0.6-1.9)	15.9 (3.4)	93.3

^aAnatomical Therapeutic Chemical classification system for Veterinary medicinal products; ^b% of the total amount of antimicrobials (in kg) used in oral group treatments

ADD= animal defined daily dose; PDD= prescribed daily dose; UDD= used daily dose

Individual antimicrobial treatment was mainly injected and only accounted for 4.2% (3.4; 2.8; 1.3-11.9%) and 2.1% (1.9; 1.5; 0.5-7.3) of the overall treatment incidence, based on ADD and UDD, respectively. Also for the individually administered antimicrobial drugs the average TI_{ADD} (= 14.8 (9.4; 12.3; 5.9-35.1)) was larger than in reality administered (TI_{UDD} = 7.6 (4.1; 7.7; 2.5-14.7)), indicating on average overdosing (Tables 3 and 4). Florfenicol, amoxicillin and the combination of lincomycin with spectinomycin were the most frequently used individually administered antimicrobials with respective proportional treatment incidences of 19.0%, 15.1% and 12.2% (Table 5). UDD/ADD ratios for the individually administered antimicrobial drugs are given in table 3.

Table 3. Daily dosages (mg/kg) and dosing ratio of individual antimicrobial treatments in white veal calves, 2007-2009 (10 cohorts, 3519 calves, Belgium)

Active substance	ATCvet ^a	ADD	UDD			UDD/ADD ratio			Total use in kg (%) ^b	Frequency of use (% of cohorts)
			Min	Mean±SD	Max	Min	Mean±SD	Max		
Procaine benzylpenicillin	QJ01CE	15	17.89	26.39±6.77	34.35	1.19	1.76±0.45	2.29	0.23 (6.9)	40
Procaine benzylpenicillin-neomycin	QJ01RC	10	10.97	21.82±9.43	35.71	1.10	2.18±0.94	3.57	0.20 (5.8)	60
Procaine benzylpenicillin-dihydrostreptomycin	QJ01RC	10	22.55	33.84±10.25	42.55	2.26	3.38±1.02	4.26	0.16 (4.7)	30
Ampicillin	QJ01CA	15		40.68			2.71		<0.01 (0.1)	10
Amoxicillin LA	QJ01CA	15	12.98	18.18±3.41	22.12	0.87	1.21±0.23	1.47	0.38 (11.2)	70
Amoxicillin-clavulanic acid	QJ01CA	7	17.28	17.71±0.61	18.14	2.47	2.53±0.09	2.59	0.02 (0.7)	20
Ceftiofur	QJ01DA	1.5	1.11	2.56±1.69	5.55	0.74	1.71±1.12	3.70	0.04 (1.2)	70
Cefquinome	QJ01DA	2	2.57	4.07±1.19	6.39	1.29	2.03±0.60	3.19	0.06 (1.9)	80
Tilmicosin LA	QJ01FA	10	6.62	12.40±6.80	25.86	0.66	1.24±0.68	2.59	0.04 (1.3)	60
Tilmicosin ^c	QJ01FA	20	16.71	19.74±4.29	22.77	0.84	0.99±0.21	1.14	0.21 (6.3)	60
Tulathromycin	QJ01FA	2.5	3.39	4.44±1.59	6.26	1.36	1.78±0.6	2.51	0.01 (0.2)	30
Lincomycin-spectinomycin	QJ01FF	15	7.17	10.12±2.33	15.26	0.48	0.67±0.16	1.02	0.34 (10.2)	90
Gentamicin	QJ01GB	3.75	4.10	5.53±1.23	7.95	1.09	1.47±0.33	2.12	0.05 (1.6)	70
Paromomycin		14	24.47	25.27±1.12	26.06	1.75	1.80±0.08	1.86	0.12 (3.4)	20
Trimethoprim-sulphonamides	QJ01EW	15	17.47	28.0±8.52	38.10	1.16	1.87±0.57	2.54	0.01 (0.3)	50
Flumequine ^c	QJ01MA	12		10.61			0.99		< 0.01 (0.1)	10
Enrofloxacin	QJ01MA	2.5	3.37	5.27±1.55	7.38	1.35	2.11±0.62	2.95	0.05 (1.4)	50
Difloxacin	QJ01MA	2.5	1.92	3.40±1.06	4.34	0.77	1.36±0.42	1.74	0.01 (0.3)	40
Marbofloxacin	QJ01MA	2.5	3.93	9.90±3.70	13.73	1.57	3.96±1.48	5.49	0.08 (2.4)	50
Danofloxacin LA	QJ01MA	2.5	9.31	14.92±4.33	21.37	3.72	5.97±1.73	8.55	0.11 (3.1)	70
Florfenicol LA	QJ01BA	10	10.63	23.64±10.24	47.06	1.06	2.36±1.02	4.71	1.23 (36.5)	100
Colistin	QJ01XB	5	4.23	5.17±0.82	5.72	0.85	1.03±0.16	1.14	0.01 (0.4)	30

^aAnatomical Therapeutic Chemical classification system for Veterinary medicinal products; ^bOnly individually administered antimicrobial group treatments; ^cOrally administered, individually used; LA= long acting; SD= standard deviation; ADD= animal defined daily dose; UDD= used daily dose

For the individual administrations no detailed prescriptions of the veterinarian were available and therefore the PDD could not be calculated. Overall, 81.8% (18/22) of the individually used antimicrobial formulations were overdosed, 13.6% was norm-dosed and 4.5% was underdosed. Only lincomycin with spectinomycin was systematically underdosed (Table 3). On 40% (4/10) of the detailed monitored cohorts none of the individual treatments were administered by the veterinarian. Veterinarians mainly administered fluoroquinolones, long acting macrolides and trimethoprim-sulphonamides. Cephalosporins were for 98.0% administered by the producers. The total antimicrobial treatment incidence (group + individual treatments (available for 10 production cohorts)) was 416.8 (148.4; 425.1; 123.4-818.9) and 387.0 (120.5; 373.7; 158.7-585.1) based on ADD and UDD, respectively. All together an average of 16 (2.1; 16.5; 13-19) different antimicrobial molecules per production cohort were used, of which on average 6 (1.9; 6.0; 5-8) were used in oral group treatments and 10 (1.9; 10.5; 7.0-13.0) in the individual treatments.

Table 4. Correctness of dosing in antimicrobial group treatments in white veal calves by indication, expressed as UDD/ADD ratio, 2007-2009 (15 cohorts, 5853 calves, Belgium)

Active substance	ATCvet ^a	Indication mean±SD (min.-max.)					
		Arrival prophylaxis	Respiratory disease	Diarrhea	Dysbacteriosis	Idiopathic peritonitis	Enterotoxaemia
Amoxicillin	QJ01CA	2.2±0.3 (1.7-2.7)	0.8±0.5 (0.4-1.4)			1.2±0.5 (0.5-1.6)	0.7
Colistin	QJ01XB	1.1±0.3 (0.5-1.6)	0.6			0.7±0.5 (0.3-1.1)	
Flumequine	QJ01XB			1.0±0.4 (0.3-1.6)		1.3±0.7 (0.8-1.8)	
Oxytetracycline	QJ01AA		1.1±0.7 (0.2-2.5)		0.4±0.5 (0.1-1.4)	0.3	
Doxycycline	QJ01AA		0.8±0.4 (0.4-1.8)				
Trimethoprim-Sulphonamides	QJ01EW	1.1±0.8 (0.2-1.8)	1.3±0.8 (0.8-1.9)				
Enrofloxacin	QJ01MA			0.7			
Ampicillin	QJ01CA					1.5	
Tilmicosin	QJ01FA		1.0				
Tylosin	QJ01FA	1.5	1.1±0.4 (0.4-1.9)		0.4±0.2 (0.1-0.6)		0.3

^aAnatomical Therapeutic Chemical classification system for Veterinary medicinal products; SD= standard deviation; ADD= animal defined daily dose; UDD= used daily dose

Table 5. Group and individual antimicrobial drug use in white veal calves, expressed as animal daily doses per 1000 calves at risk per day, 2007-2009, Belgium

Active substance	ATC vet ^a	TI _{ADD} ^b			TI _{PDD} ^b			TI _{UDD} ^b			Prop. TI _{ADD} % ^c	Prop. TI _{PDD} % ^c	Prop. TI _{UDD} % ^c
		Min	Mean±sd	Max	Min	Mean±sd	Max	Min	Mean±sd	Max			
Group treatments^e													
Amoxicillin	QJ01CA	0	129.0±67.6	310.6	0	73.4±39.3	163.1	0	71.8±32.0	143.6	31.2	19.4	18.5
Ampicillin	QJ01CA	0	1.5±5.9	22.8	0	0.8±3.2	12.3	0	1.0±3.8	14.8	0.4	0.2	0.3
Tylosin	QJ01FA	0	58.4±44.3	138.3	0	71.3±55.9	166.6	0	66.6±56.4	201.0	14.1	18.8	17.2
Tilmicosin	QJ01FA	0	1.0±4.0	15.3	0	1.2±4.6	17.8	0	1.1±4.1	15.9	0.3	0.3	0.3
Trimethoprim-Sulfonamides	QJ01EW	0	17.6±31.1	98.6	0	13.2±22.1	64.7	0	15.0±23.1	56.9	4.2	3.5	3.9
Oxytetracycline	QJ01AA	0	79.3±57.8	212.4	0	81.7±46.5	143.4	0	91.5±59.1	200.0	19.2	21.5	23.7
Doxycycline	QJ01AA	9.1	35.8±22.2	81.6	12	45.4±35.6	124.5	24.5	46.7±31.7	119.6	8.6	12.0	12.1
Flumequine	QJ01XB	0	34.1±25.0	82.6	0	33.8±25.6	82.2	0	34.1±24.7	85.1	8.2	8.9	8.8
Enrofloxacin	QJ01MA	0	0.2±0.9	3.5	0	0.3±1.3	5.2				0.1	0.1	0.1
Colistin	QJ01XB	0	57.1±22.4	96.7	0	57.9±24.9	114.7	0	58.9±38.2	180.9	13.8	15.3	15.2
Individual treatments^f													
Procaine benzylpenicillin	QJ01CE	0	0.29±0.52	1.33				0	0.18±0.30	0.77	2.0		2.3
Procaine benzylpenicillin+ neomycin	QJ01RC	0	0.77±0.90	2.44				0	0.50±0.77	2.41	5.2		6.6
Procaine benzylpenicillin + dihydrostreptomycin	QJ01RC	0	0.43±0.92	2.81				0	0.16±0.40	1.27	2.9		2.2
Ampicillin	QJ01CA	0	0.01±0.04	0.14				0	<0.01±0.02	0.05	0.1		0.1
Amoxicillin LA	QJ01CA	0	1.26±1.42	4.29				0	1.15±1.48	4.72	8.5		15.1
Amoxicillin + clavulanic acid	QJ01CA	0	0.07±0.20	0.64				0	0.03±0.08	0.25	0.5		0.4
Ceftiofur	QJ01DA	0	0.39±0.84	2.73				0	0.21±0.32	0.91	2.6		2.8
Cefquinome	QJ01DA	0	0.83±1.02	2.95				0	0.44±0.53	1.62	5.6		5.2
Tilmicosin LA	QJ01FA	0	0.66±0.86	2.55				0	0.55±0.73	2.35	4.4		7.2
Tilmicosin ^d	QJ01FA	0	0.16±0.35	0.90				0	0.18±0.38	1.02	1.1		2.4
Tulathromycin LA	QJ01FA	0	0.45±0.82	2.09				0	0.25±0.44	1.20	3.0		3.3
Lincomycin + spectinomycin	QJ01FF	0	0.53±0.51	1.55				0	0.92±1.00	3.30	3.6		12.2
Gentamicin	QJ01GB	0	0.48±0.69	2.02				0	0.32±0.48	1.44	3.2		4.2
Paromomycin		0	0.27±0.84	2.65				0	0.14±0.45	1.42	1.8		1.9
Trimethoprim + sulphonamides	QJ01EW	0	0.09±0.11	0.31				0	0.05±0.07	0.18	0.6		0.7
Flumequine ^d	QJ01MA	0	<0.01±0.01	0.02				0	<0.01±0.01	0.02	<0.1		<0.1
Enrofloxacin	QJ01MA	0	0.47±0.80	2.26				0	0.20±0.32	0.81	3.2		2.7
Difloxacin	QJ01MA	0	0.19±0.51	1.62				0	0.11±0.29	0.91	1.3		1.4
Marbofloxacin	QJ01MA	0	0.93±1.17	3.07				0	0.31±0.55	1.8	6.3		4.1
Danofloxacin LA	QJ01MA	0	2.04±3.62	11.50				0	0.33±0.60	1.94	14.1		4.4
Florfenicol	QJ01BA	0.2	4.38±6.70	21.32				0.13	1.44±1.58	4.22	30.3		19.0
Colistin	QJ01XB	0	0.13±0.37	1.18				0	0.15±0.43	1.37	0.9		2.0

^aAnatomical Therapeutic Chemical classification system for Veterinary medicinal products; ^bTreatment incidences based on ADD= animal defined daily dose, PDD= prescribed daily dose or UDD= used daily dose; ^cProportional TI_{ADD}, TI_{PDD} and TI_{UDD}; ^dOrally administered, individually used; ^eData available for 15 production cohorts, 5853 calves; ^fData available for 10 production cohorts, 3519 calves; LA= long acting; SD= standard deviation

Anti-inflammatory drugs were far less frequently used compared to antimicrobials ($TI_{UDD} = 5.94$). Only sodium salicylic acid was used in group treatments to prevent shock after parenteral administration of iron dextran (13/17) and to treat respiratory disease (4/17 treatments). The main indication for individual use of (N)SAID's was respiratory disease. Treatment incidences of the individually used (N)SAID's are given in table 6. Most administrations of NSAID's were administered by the producers, whereas steroidal were exclusively administered by the veterinarian. Orally administered sodium salicylic acid was underdosed, whereas the individually injected (N)SAID's were generally overdosed (Table 6). Other non-antimicrobial drugs used in the followed cohorts, included group treatments with pour on formulations of macrocyclic lactones to treat scabies (exclusively BB) or lice and individual administration of macrocyclic lactones, vitamin preparations, diclazuril and halofuginone.

Table 6. Use and correctness of dosing of (non)-steroidal anti-inflammatory drugs in white veal production in Belgium, 2007-2009

Active substance	ADD (mg/kg)	UDD (mg/kg)	TI_{ADD}^a	TI_{UDD}^a	UDD/ADD ratio	Frequency of use
			Mean±SD (min-max)			(% of cohorts)
<u>Group treatments^b</u>						
Sodium salicylic acid	40	27.62±9.53 (17.62-43.81)	3.43±2.74 (0-6.85)	5.42±4.83 (0-15.54)	0.69±0.24 (0.44-1.10)	66.7
<u>Individual treatments^c</u>						
Dexamethason	0.06	0.15±0.05 (0.07-0.23)	0.45±0.81 (0-2.49)	0.17±0.30 (0-0.91)	2.57±0.90 (1.18-3.78)	60.0
Flunixin meglumin	2.2	2.84±1.33 (1.47-4.55)	0.13±0.18 (0-0.57)	0.13±0.20 (0-0.65)	1.29±0.61 (0.67-2.07)	70.0
Ketoprofen	3.0	4.31±1.75 (2.95-7.25)	0.71±1.60 (0-5.12)	0.36±0.66 (0-1.96)	1.44±0.58 (0.98-2.42)	50.0
Meloxicam	0.5	0.98±0.35 (0.53-1.63)	0.57±0.81 (0-2.46)	0.38±0.57 (0-1.50)	1.96±0.70 (1.07-3.25)	80.0
Sodium metamizole	40	44.85±10.94 (30.20-62.65)	0.23±0.37 (0-1.13)	0.20±0.30 (0-0.87)	1.12±0.27 (0.76-1.57)	60.0
Total individual treatments ^c			2.10±2.05 (0.10-6.39)	1.24±1.06 (0.13-2.80)		
<u>Total all treatments^c</u>			5.11±3.57 (0.22-11.03)	5.94±5.42 (0.15-18.20)		

^aTI= treatment incidence expressed as number of calves treated daily per 1000 calves at risk ^bData available for 15 production cohorts, 5853 calves; ^cData available for 10 production cohorts, 3519 calves; SD= standard deviation

STATISTICAL ANALYSIS

Univariable testing delivered 6 significant risk factors for antimicrobial use (TI_{UDD}) at the $P < 0.2$ level: season, veterinarian, integrator, integration size, herd location and region, of which the latter 5 were highly correlated. Of these, only the most significant variable, namely integration size was included in the multivariable model. The size of the integration was the only variable to remain marginally significant in the final multivariable model, with cohorts from smaller integrations using a larger amount of antimicrobials in group treatments ($P < 0.05$). The average TI_{UDD} was 350.6 and 487.1 UDD per 1000 calves in herds belonging to large and small integrations, respectively.

Univariable testing for the UDD/ADD ratio delivered two significant variables, namely the number of caretakers and total mortality. Both variables remained significant in the final multivariable model. Cohorts with a single caretaker overdosed on average, compared to cohorts with more than one caretaker, which norm-dosed ($P < 0.01$). The total mortality was a significant predictor ($P < 0.05$). Cohorts with low mortality (<3%) tended to overdose, but no distinguishment between the three classes could be made due to lack of power.

DISCUSSION

Since not all drugs delivered on a farm are used and because treatment practices are not always in compliance with the manufacturers prescriptions, the most accurate information on drug use is obtained by monitoring the end-users (Nicholls et al., 2001; Timmerman et al., 2006; van der Fels-Klerx et al., 2011). Monitoring practices at the level of the end-user can provide essential information on what aspects of responsible antimicrobial use are specifically needed to be trained within a certain sector. In contrast to total drug consumption data or sales, provided by pharmaceutical companies or large distributors, collection of end-user data is more laborious and expensive and therefore more likely to result in insufficient data (Nicholls et al., 2001). Since retrospective data collection is subject to recall bias, prospective data collection was used. For these reasons cohorts with well-motivated producers were conveniently selected in the present study, possibly leading to selection bias (Thrusfield, 1996). Because the sample size included 5% of the population (all breeds included) with more than 90% of veterinarians and integrations active in the field represented, and because

housing and feeding of white veal calves are highly standardized in Belgium, this bias is believed to be limited. Despite the drawbacks of convenience selection, the sample is assumed to be representative for the Belgian veal industry at present.

The incidence of group antimicrobial treatments ($TI_{ADD} = 414.0$ ADD per 1000 veal calves) was strikingly higher than in conventional dairy and beef cattle (6.3 and 5.4 per 1000 cattle, respectively), pigs (178.1 per 1000 pigs in 2003 and 235.7 in 2010) or poultry (121.4 per 1000 chickens) in Belgium (Timmerman et al., 2006; Catry et al., 2007b; Callens et al., 2011; Persoons, 2011). However, the treatment incidence based on a standard weight of a white veal calf of 164 kg as used in the Dutch MARAN report, was much lower than the TI based on the actual live weight, namely 164.3 ADD per 1000 calves (MARAN-2009, 2011). The best estimation of the treatment incidence is obtained by calculating the number of daily dosages on the basis of the best possible estimate of the average live weight at the time of treatment (MARAN-2009, 2011), as was done in the present study. Due to a large variation in body weight according to age and breed in different production animal species, an applied body weight can seriously influence the calculated TI's, as was demonstrated (Figure 2). Another issue is what daily dose to use for the calculation of TI's. As the PDD and UDD tend to vary over time and in between veterinarians and producers, the use of an internationally agreed ADD for animals is advisable in order to compare different production systems or countries. The selected ADD's influence the calculated TI's and therefore should at least be mentioned in each report.

Despite these comments on methodology, antimicrobial use in the Belgian veal industry was still twice as high compared to the Netherlands, as has been reported for pigs and poultry (Timmerman et al., 2006; Persoons, 2011). However, it is important to remark that the reported TI's for the Dutch veal industry imply both 'white' (70%) and 'rosé' veal (30%). Since rosé veal systematically receives less antimicrobials than white veal due to a different nutritional management (ruminating calves), the antimicrobial use in white veal in the Netherlands is probably higher than reported for all veal types in the MARAN report. The most likely explanation for the higher antimicrobial drug use in veal calves compared to poultry and pigs, is the typical organization of the veal industry. Whereas pig and poultry herds are mainly closed or only combine animals from a limited number of origins, the veal industry commingles young, recently transported,

highly stressed calves which originate from multiple farms, both domestic and foreign. The combination of these factors is known to cause a higher disease risk (Cusack et al., 2003; Taylor et al., 2010).

All group treatments in veal calves were orally administered. Main reasons for the preference for oral administrable antimicrobials in the veal industry are the easy administration in the milk during the feeding routine and the low cost per calf. In contrast, a good application of individual injections requires continuous visual inspection of the calf and compliance with the prescribed treatment length, both of which are very laborious and require well trained producers. Another important issue with individual treatment in the present study was the large number of different molecules used per cohort, including the widespread use of so defined *critically important* cephalosporins, fluoroquinolones, penicillins and macrolides (WHO, 2007). Veterinarians still tended to administer fluoroquinolones and long-acting macrolides themselves, but the highest proportion of these molecules was administered by the producers as well. The fact that 3rd and 4th generation cephalosporins were almost exclusively administered by the producers in all monitored cohorts is worrisome, but similar to the situation in North American dairy farms (85%) (Pol and Ruegg, 2007). In contrast, in Canadian cow-calf herds and American beef cattle the percentage of herds on which cephalosporins were used was much lower (16% and 3.8%, respectively) (Gow and Waldner, 2009; Green et al., 2010). Despite the highly variable individual use, the most frequently used individually administered drugs, namely florfenicol and lincomycin/spectinomycin are not classified as critical for human medicine, opening perspectives for injectable formulations (WHO, 2007).

The overall TI_{UDD} was smaller than the overall TI_{ADD} , suggesting overdosing. Nevertheless, more than 40% of the group antimicrobial treatments were still underdosed as observed in pigs (Timmerman et al., 2006; Callens et al., 2011). Only amoxicillin as arrival prophylaxis was markedly overdosed. The systematic severe underdosing of oxytetracycline and tylosin when used to treat dysbacteriosis, was a striking finding. An overestimation of the bodyweight for amoxicillin at arrival and an underestimation of the body weight in the second half of the production cycle when dysbacteriosis is most frequently treated, seems the most likely explanation. However, the UDD/PDD ratio of the oral group treatments showed that producers tended to

closely follow the veterinarian's prescriptions. In fact, for dysbacteriosis, lower antimicrobial dosages than for the main indication (=respiratory disease) were prescribed, as was the case for enterotoxaemia. Macrolide and oxytetracycline resistances were the most frequently detected resistances in Pasteurellaceae in white veal calves in Belgium, and this is possibly related to these underdosing practices for dysbacteriosis (Catry et al., 2005; Pardon et al., 2011). Why prescriptions by the veterinarian were overall slightly overdosed in cohorts with a single caretaker, whereas they were correctly followed in cohorts with multiple caretakers is not completely understood. Since there was no correlation of the number of caretakers with the herd size or integration, the explanation most probably lies in other, likely socioeconomic, aspects of a more professional approach of veal farming in herds with multiple caretakers. The trend towards higher mortalities in underdosing cohorts, is most likely caused by the deliberate underdosing on cohorts with high mortalities due to enterotoxaemia.

Perhaps the most exploitable finding in the present study was the trend that larger integrations used less antimicrobials. Since integration size, integration, veterinarian and region were highly correlated, this signifies an important influence of the integration as a whole on antimicrobial consumption in veal herds. It is clear that reduction of antimicrobial drug use in the veal industry can only be achieved by reducing the number of oral antimicrobial group treatments, since this implies a high degree of metaphylactic treatments (87%). In the Netherlands, where the production system and diseases are highly similar to Belgium, a significant reduction in antimicrobial use in veal calves has already been achieved (MARAN-2009, 2011). Previous studies have shown that good economical results can be obtained with metaphylactic injection therapy with long acting formulations only, both at arrival as when a clinical outbreak occurred (Van Donkersgoed, 1992; Booker et al., 2007; Catry et al., 2008; Baggott et al., 2011). Also, correct dosing, instead of overdosing, is an option to reduce antimicrobial use. Because of the multifactorial nature of respiratory disorders in cattle, not only improved treatment protocols but also vaccination, correct housing, disinfection, and preconditioning of the calves might further reduce antimicrobial use (Sweiger and Nichols, 2010). The use of (N)SAID's in veal calves is currently limited. Including NSAID's in standard individual treatment protocols could enhance both production results as animal welfare (Bednarek et al., 2003a,b). However, attention

should be given not to overdose NSAID's, because of the possible toxic side effects, especially in dehydrated animals, such as diarrheic calves and in case of the prolonged use of sodium salicylic acid as a group treatment (Yeomans et al., 2009; Bardou and Barkun, 2010). In addition to these measures, continuous monitoring of group and individual antimicrobial use with an electronic recording system (personal digital assistant) is recommended to guarantee accurate data input (DeVincent and Reid-Smith, 2006; Gonzalez et al., 2010).

CONCLUSIONS

The present study offers a benchmark for antimicrobial use in the European veal industry, based on the Belgian situation. At present, antimicrobial drug use is intensive and highly variable. Several opportunities to reduce antimicrobial use should be evaluated in future studies. Reduction of antimicrobial use in the veal industry is a joined responsibility, of which the initiative lies with the integration.

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TRANSPARENCY DECLARATIONS

None to declare

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CHAPTER 4

MORBIDITY AND MORTALITY

IN WHITE VEAL CALVES

LONGITUDINAL STUDY ON MORBIDITY AND MORTALITY IN WHITE VEAL CALVES IN BELGIUM

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ABSTRACT

Background: Mortality and morbidity are hardly documented in the white veal industry, despite high levels of antimicrobial drug use and resistance. The objective of the present study was to determine the causes and epidemiology of morbidity and mortality in dairy, beef and crossbred white veal production. A total of 5853 calves, housed in 15 production cohorts, were followed during one production cycle. Causes of mortality were determined by necropsy. Morbidity was daily recorded by the producers.

Results: The total mortality risk was 5,3% and was significantly higher in beef veal production compared to dairy or crossbreds. The main causes of mortality were pneumonia (1.3% of the calves at risk), ruminal disorders (0.7%), idiopathic peritonitis (0.5%), enterotoxaemia (0.5%) and enteritis (0.4%). Belgian Blue beef calves were more likely to die from pneumonia, enterotoxaemia and arthritis. Detection of bovine viral diarrhoea virus at necropsy was associated with chronic pneumonia and pleuritis. Of the calves, 25.4% was treated individually and the morbidity rate was 1.7 cases per 1000 calf days at risk. The incidence rate of respiratory disease, diarrhoea, arthritis and otitis was 0.95, 0.30, 0.11 and 0.07 cases per 1000 calf days at risk, respectively. Morbidity peaked in the first three weeks after arrival and gradually declined towards the end of the production cycle.

Conclusions: The present study provided insights into the causes and epidemiology of morbidity and mortality in white veal calves in Belgium, housed in the most frequent housing system in Europe. The necropsy findings, identified risk periods and differences between production systems can guide both veterinarians and producers towards the most profitable and ethical preventive and therapeutic protocols.

BACKGROUND

The white veal industry is specialized in rearing calves from different breed and origin on a low-iron milk powder diet. The veal industry is highly integrated and Europe produces about 6 million veal calves yearly, raised predominantly in France, the Netherlands, Italy and Belgium (Sans and De Fontguyon, 2009). The incidence of calf diseases differs between production systems and geographical locations and varies over time (Sivula et al., 1996b; Busato et al., 1997; Sanderson and Dargatz, 2000; Loneragan et al., 2001; Svensson et al., 2003; 2006b,c; Gulliksen et al., 2009). Therefore collection of local and temporal data is to be preferred. In contrast to conventional dairy, beef, suckler and feedlot calves, mortality and morbidity is hardly documented in veal calves. Previous studies addressed mortality in veal calves housed in individual stalls (crates) in the United States and Canada (Sargeant et al., 1994b; Stull and McDonough, 1994; Wilson et al., 1994). This housing system has been completely abandoned in Europe (Council Directives 91/629/EC and 97/2/EC) and in certain states in the United States. The only recent European study addressed a niche production system with an exceptionally high animal welfare standard in Switzerland, a minor producing country (Bähler et al., 2010). White veal production can be divided into three production systems, based upon the type of calf that is reared, namely dairy, beef or crossbred veal. These systems do not only differ according to the selected breeds, but also have a different nutritional and organizational management. Previous studies only addressed dairy calves, whereas in several countries also the other production systems are present (Sargeant et al., 1994b; Bähler et al., 2010).

No contemporary study on morbidity and mortality in the most frequent veal housing system of the European mainland, which is group housing on slatted floors in pens of 2 to 8 animals after a 6 weeks period of individual housing, is currently available. Nowadays such a study is of particular interest, since multidrug resistance is abundantly present in the veal industry and of great public concern (Catry et al., 2005b, 2007b; Di Labio et al., 2007; Graveland et al., 2010; Cook et al., 2011; Graveland et al., 2011; van Cleef et al., 2011). Additionally a recent study showed that antimicrobial use in the veal industry is highest of all food producing animals (Pardon et al., 2012). All these observations force the veal industry to evaluate current treatment protocols and search for alternative approaches. Longitudinal mortality and morbidity data provide essential

information for the understanding of current practices and their consequences in white veal calves, and can form the basis for novel preventive strategies.

Therefore, the objective of the present field study was to determine the causes and epidemiology of mortality and morbidity in white veal calves, housed within different production systems.

METHODS

STUDY DESIGN, SELECTION OF HERDS AND ANIMALS

A prospective longitudinal survey based on a sample of 5% of the Flemish veal herds was conducted to monitor all morbidity and mortality events in one production cycle per herd. The study population consisted of all veal herds in Flanders (Northern Belgium) certified by the Belgian Controlled Veal (BCV) label. The sampling frame was the list of veal herds in Flanders officially registered in the Belgian cattle registration system (SANITEL, Animal Health Service-Flanders). Of the 295 herds in Belgium, 285 herds (97%) are situated in Flanders and 271 herds (95%) complied with the BCV label. Because of the intensive registration, the routine visiting and reporting necessary, farms were conveniently selected. Selection criteria included the willingness to keep detailed registration records on diseases and treatment and allowing the use of farm data. Selection was independent of any disease history and for logistic reasons the farms were gradually initiated in the study over a 2 year period. A production cohort was defined as one all in all out production cycle, which lasted from arrival to slaughter (6-8 months). The study group consisted of 15 production cohorts, in 15 herds. The sample was stratified on production system: dairy (n=5), crossbred (n= 5) and beef (n= 5) cohorts.

DATA COLLECTION

REGISTRATION OF MORTALITY DATA AND DEFINITIONS

All calves were individually identified by ear tag, according to Belgian law. Calf arrival data were collected from the Belgian cattle registration system (SANITEL- Animal Health Service-Flanders). The reasons for calves not finishing the production cycle were death, culling (= unwanted early slaughter) or transfer to another production system. The latter category included calves which were unable to adapt to the intensive milk diet or

the concentrate replacer diet and were removed from the veal stables to be fattened as conventional calves. Unwanted early slaughter was defined as calves being individually slaughtered before the rest of the group, mostly for reasons of trauma or sudden respiratory symptoms. Calf identity, mortality date and preceding symptoms were recorded on registration forms by the producers. A gross postmortem examination of the animals which died during production was performed either on farm by a specialized veterinarian or at the Animal Health Service-Flanders. For these postmortem examinations a standardized protocol was always followed. For data processing only one cause of mortality was registered. If more than one lesion was present, the most severe lesion was used as reason of death. If an obvious reason for the animals death was known from the anamnesis (e.g. shock as a consequence of parenteral iron administration) and the autopsy findings complied with this diagnosis, the animal was classified as such. The group of respiratory diseases included two gross diagnoses: pneumonia and necrobacillosis (laryngeal diphtheria). Pneumonia was only held responsible for the animals death if more than one third of the lung was affected, and other lethal lesions were absent. Also pneumonia cases with concurrent pleuritis or pericarditis but without peritonitis (see definition polyserositis underneath) were classified as pneumonia cases. Calves which suffocated as a consequence of air way blockage by necrotic lesions upon the vocal cords/arytenoids were classified as necrobacillosis. The group of digestive diseases included acute ruminal disorders, enteritis, enterotoxaemia, mesenteric torsion, intussusception, liver disease, abomasal hemorrhage and peritonitis due to perforating abomasal ulceration. Ruminal disorders included all acute ruminal pathology (frothy bloat and acute ruminal acidosis/rumenitis) causing sudden death. Cases were classified as enteritis when a macroscopic enteritis and obvious smearing of the hind legs were present at necropsy. Enterotoxaemia was defined as the presence of an extensive necrohaemorrhagic enteritis. Calves were classified as idiopathic peritonitis if at least a peritonitis, without an obvious internal cause (e.g. perforating abomasal ulceration, intussusception,...) was present. This group included cases in which besides a peritonitis also pericarditis and pleuritis were present (polyserositis). Liver disease included hepatitis, severe hepatic steatosis and generalized icterus. Cases in which the liver was involved in an omphaloflebitis were classified as omphalitis cases, together with omphalitis as such, omphalo-urachitis, omphaloarteritis and umbilical abscesses. Septicemia as such was

not determined, and cases which presumably died due to septicemia were classified according to the major lesion (omphalitis, enteritis, meningitis or pneumonia). The group of neurological disorders included hydranencephalia, hydrocephalus and meningitis. The group of orthopedic diseases included euthanasia due to severe arthritis, limb or vertebral column fractures and death due to accidental hanging. Only when necropsied at the Animal Health Service hearts were examined and calves with congenital heart defects were classified as such. Calves which died suddenly and did not show obvious findings of any of the above mentioned acute diseases at necropsy, besides a mild (hemorrhagic) enteritis, were classified as sudden death of unknown origin. Calves which were dead on arrival or died the first day after arrival, were classified as dead on arrival. Calves that were not autopsied (e.g. due to extensive postmortal decay), were classified as such.

DETAILED NECROPSIES AND ADDITIONAL VIROLOGICAL AND BACTERIOLOGICAL INVESTIGATIONS

For calves necropsied at the Animal Health Service, histopathology was performed in case no gross diagnosis was possible. Additionally, samples for bacteriological and virological examination were taken. In cases of neonatal enteritis a commercial antigen ELISA for *Cryptosporidium parvum*, bovine coronavirus, bovine rotavirus and *Escherichia coli* (F5) was performed on intestinal content (Digestive ELISA kit, Bio-X, Jemelles, Belgium). Isolation of *Salmonella spp.* was attempted in cases with suspicious lesions, by aerobically culturing intestinal content on brilliant green agar plates (Lab-M, Bury, UK). Isolation of respiratory bacteria (Pasteurellaceae and *Mycoplasma spp.*) from pneumonia lesions was performed according to standard protocols, described elsewhere (Catry et al., 2007a; Catry et al., 2008; Pardon et al., 2011). The presence of bovine viral diarrhoea virus (BVDV) was examined by PCR on spleen tissue (Letellier and Kerkhofs, 2003). For selected cases of acute pneumonia PCR analysis for bovine herpesvirus 1 (BHV-1) (Abril et al., 2004) and bovine respiratory syncytial virus (BRSV) (Boxus et al., 2005) together with virus isolation for bovine adenovirus 3 (BAV-3) and parainfluenzavirus type 3 (PI-3) was performed at the Veterinary and Agrochemical Research Centre (CODA-CERVA, Ukkel, Belgium) according to in house standard protocols, described elsewhere (Pardon et al., 2011).

REGISTRATION OF MORBIDITY DATA AND DEFINITIONS

Morbidity was estimated on the bases of individual treatment of the calves. A calf was considered a case of a given disease, when treated individually for that indication on at least one day by the producer or veterinarian. This treatment included both single or multiple, antimicrobial or non-antimicrobial drugs. An initial case was defined as the first treatment of a calf for a given indication. A reoccurrent case was defined as a calf receiving a new treatment for the same indication more than 5 and less than 15 days after the last treatment for that indication. A relapse case was defined as a calf receiving a new treatment for the same indication more than 14 days after the last treatment for that indication (Assie et al., 2004). Data were collected on the bases of the daily recording of individual treatments by the producers on preprinted registration forms. Treatments performed by the veterinarian were also registered on the same forms. The following diagnostic reasons for individual treatment were optioned: (bovine) respiratory disease (BRD), diarrhea, idiopathic peritonitis, acute ruminal disorder, ruminal drinking, otitis, arthritis, omphalitis, laryngeal necrobacillosis, nervous symptoms and miscellaneous. Herds were visited by the primary investigator between 4 and 8 times during the registration period in order to check compliance with the recording system.

DATA MANAGEMENT AND STATISTICAL ANALYSIS

Mortality and morbidity (treatment) data were entered in a relational data base (Access 2007, Microsoft Inc., Washington, DC) and transferred to SAS version 9.1 (SAS Institute Inc., Cary, NC) for descriptive and statistical analysis. Mortality data were consistent for 5853 calves. Treatment records (morbidity) were judged as unreliable on 5 cohorts, because of inconsistencies with calf identification. Therefore morbidity data was limited to 3519 calves from 10 cohorts. Mortality/morbidity risks were calculated as the number of mortalities/diseased calves over the number of calves at risk at the start of the study. Mortality/morbidity rates were calculated as the number of mortalities/diseased calves (initial and relapse) over the number of calf-days-at-risk (Assie et al., 2004; Dohoo et al., 2009). Reoccurrent cases (within 14 days after initial treatment) were considered as a failure of initial treatment and therefore not included in the calculations of morbidity risks and rates. For mortality a calf was considered at

risk when present alive in the cohort. For morbidity a calf was considered at risk when present alive in the cohort and not individually treated in the past 14 days for the indication of interest (Assie et al., 2004). In this respect days spent on oral group antimicrobial treatments were not taken into account. The long acting effect of certain antimicrobial formulations was taken into account by counting one injection as 2 (tilmicosin, amoxicillin, florfenicol, danofloxacin) or 9 days (tulathromycin) of treatment. The proportion of reoccurrent and relapse cases was calculated by dividing the number of reoccurrent/relapse cases by the number of initial cases for each cohort. In the same way, the fatality rate was calculated as the number of fatal cases of a given disease over the number of initial cases.

Because calves are reorganized according to drinking speed several times per production cycle, analysis at the compartment and pen level was not possible. The unit of analysis was the individual calf. To analyze relationships between production system (dairy, beef or crossbred) and mortality, separate multivariable Cox proportional hazard models were built, with total mortality, pneumonia, enteritis, ruminal disorders, enterotoxaemia, idiopathic peritonitis, death at arrival, abomasal hemorrhage, perforating abomasal ulceration and arthritis as binary outcome variables. The PROC PHREG statement was used, including the positive stable frailty models in the SAS macro to account for clustering within a herd (Shu and Klein, 2005). The end of the observation period was the date of slaughter, if death had not occurred.

To analyze the effect of the production system on morbidity, separate multivariable models were fit with total morbidity, BRD, enteritis, otitis and arthritis as binary outcome variables. PROC GLIMMIX with binomial distribution and logit link function with Wald's statistics for type 3 contrasts was used with herd as random effect. Associations between the different pathological lesions (pneumonia (acute-chronic-pulmonary abscess), enteritis (catarrhal-hemorrhagic), pleuritis, pericarditis, abomasal ulceration, ruminal bloat and peritonitis) and between the lesions and additional diagnostic test results (BVDV PCR and bacteriology of lung lesions) were determined by logistic regression (PROC LOGISTIC). Significance was set at $P < 0.05$.

RESULTS

DESCRIPTION OF STUDY HERDS COMPOSITION AND MANAGEMENT

Herds entered the study between October 2007 and October 2009. A total of 5853 calves was followed (2744 on dairy cohorts, 1624 on crossbred cohorts and 1485 on beef cohorts). Calves on dairy cohorts were predominantly black and red Holstein Friesian (HF), whereas calves on beef cohorts were exclusively Belgian Blue (BB) double muscled calves. In crossbred cohorts, predominantly HFxBB crossbreds were housed. Mean herd size of the selected herds was 679 (standard deviation (SD)= 334), which was comparable to the sampling frame (Student's t-test, $P=0.22$). The average number of calves in the followed cohorts was 390 (SD=167). The sample contained calves from the three main integrators in Belgium ($n= 4797$) and from 3 smaller integrators ($n= 1056$). The 15 herds were under supervision of 5 different veterinary practices. The production cycle length was 196.4 days on average (SD= 9.2; Range (R)(min.-max.)= 174.9-211.0). Calves belonging to the same production cohort were housed in the same stable, which was divided into different compartments in all but two herds. All calves were individually housed during the first 6 weeks and thereafter group housed in galvanized pens on slatted floors. The diet was different between the three production systems. Dairy calves started on a 50/50 ratio with skimmed milk powder and so called nil product (whey and vegetable proteins), which changed to 100% nil product at 8 weeks post arrival on average. Crossbreds received higher quality skimmed milk powder in the first weeks, but eventually also reached a 100% nil product diet. On the contrary, beef calves never reached 100% nil product, and predominantly received high quality skimmed milk powder. In addition, concentrates and fibers were provided in each production system. Calves were not vaccinated against any pathogen.

DESCRIPTIVE EPIDEMIOLOGY OF MORTALITY

Overall, 308 calves (5.3%) died during production and 0.3% was unwanted early slaughtered. Unwanted early slaughter only occurred on 3 beef cohorts, ranging from 0.6 to 3.8% of the calves. Of the calves that died, on average 82.3% (253/308) was necropsied, ranging from 47.1-100.0% at the cohort level. The main reason for not necropsying a calf was the producer neglecting to timely inform the veterinarian, resulting in too advanced postmortal decay for interpretation. The non-necropsied calves also included the calves which were classified as 'death at arrival' (3.6% (11/308)) for the same reason. Overall, the digestive system accounted for 41.9% of mortality, the respiratory system for 27.7%, the musculoskeletal system for 3.6%, the nervous system for 2.0% and idiopathic peritonitis as such for 14.6%.

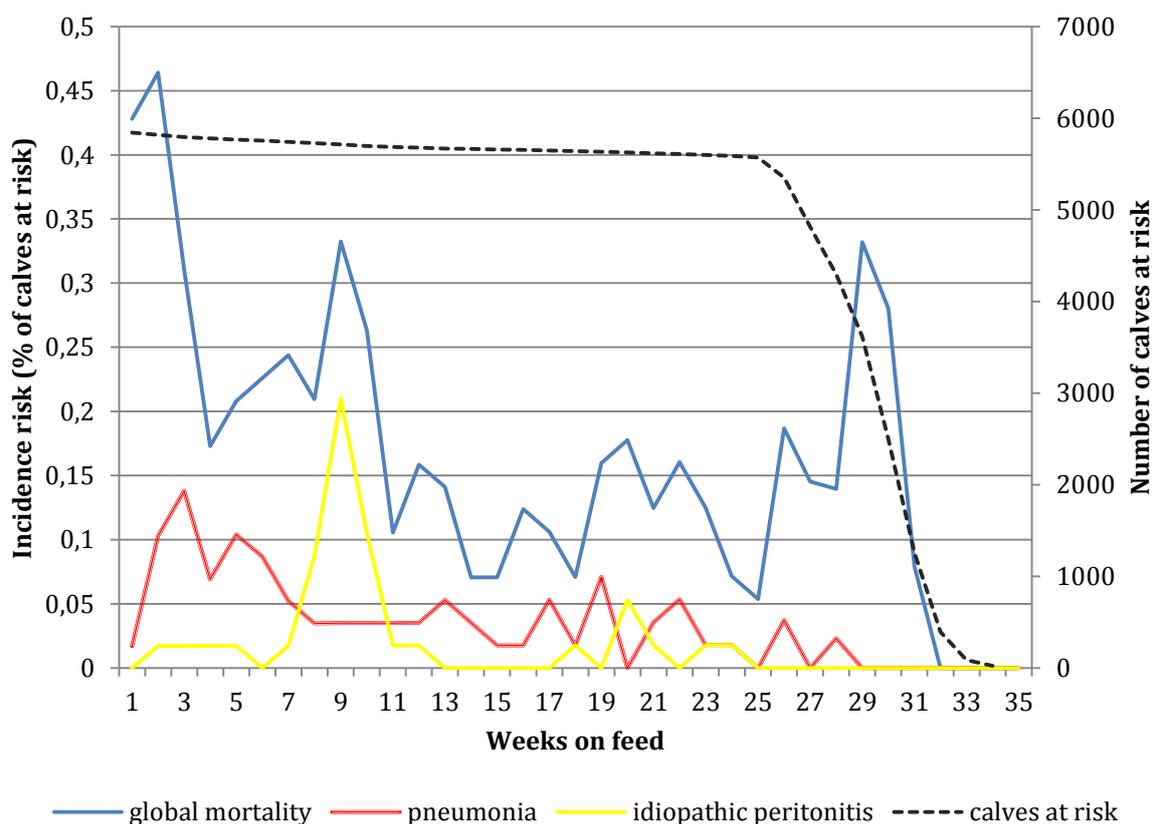


Figure 1. Mortality risk (%) of pneumonia and idiopathic peritonitis according to week on feed in 5853 white veal calves, housed in 15 cohorts in Belgium (2007-2009).

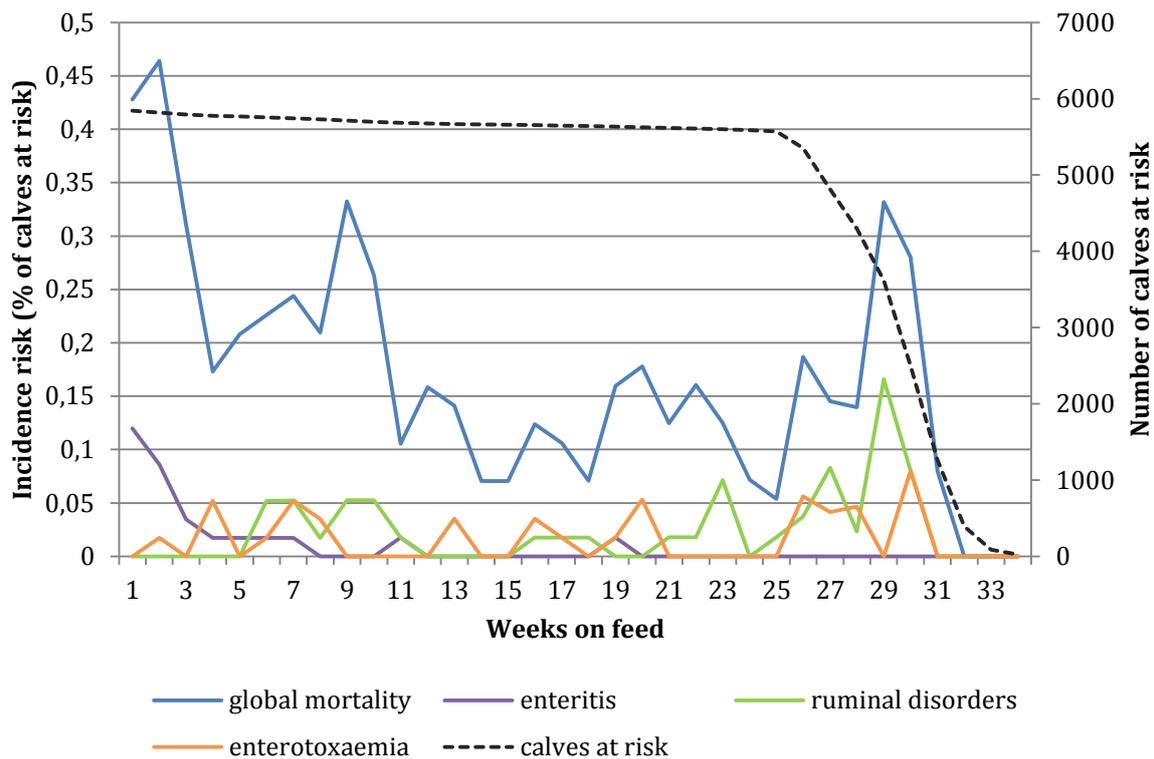


Figure 2. Mortality risk (%) of selected digestive diseases according to week on feed in 5853 white veal calves, housed in 15 cohorts in Belgium (2007-2009).

The leading individual causes of mortality were pneumonia (on average 27.0% of the losses at the cohort level; SD= 14.4; R= 7.7-62.5), ruminal disorders (18.6%; SD= 14.4; R= 0-44.4), idiopathic peritonitis (11.9%; SD= 14.3; R= 0-51.5), enterotoxaemia (10.0%; SD= 10.6; R= 0-38.5) and enteritis (9.6%; SD= 8.3; R= 0-25.0%). Mortality risks and rates for the different causes of mortality are listed by production system in table 1. Overall, the mortality risk was highest in the first weeks after arrival, gradually declined until week 12 and increased again at the end of the production cycle (Figures 1 and 2). Overall, mortality was higher in beef cohorts compared to dairy (hazard ratio (HR)= 1.6; 95% confidence interval (CI)= 1.0-2.5; $P<0.05$) or crossbred cohorts (HR= 2.3; CI 1.5-3.9; $P<0.01$) (Figure 3). Three major peaks in total mortality could be identified (Figures 1 and 2). The first and highest one occurred at week two and in that week the most important causes of mortality were pneumonia (27.3%), enteritis (22.7%), hydranencephalia (13.6%) and omphalitis (13.6%). Mortality due to pneumonia peaked between week 2 and 6, but continued at lower level throughout the entire cycle (Figure 1).

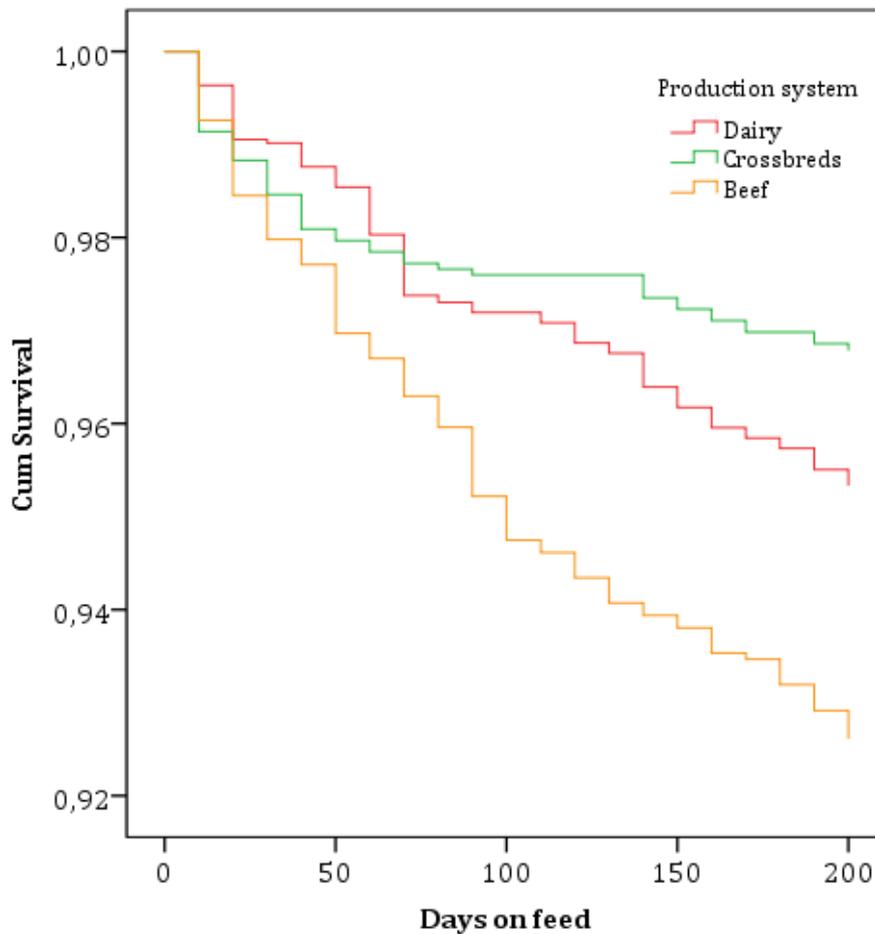


Figure 3. Survival distribution function for mortality in white veal calves by production system (15 cohorts, 5853 calves, 2007-2009, Belgium).

Calves housed in beef cohorts were more likely to die from pneumonia compared to dairy calves (HR= 2.5; CI= 1.1-5.8; $P<0.05$) and crossbreds (HR= 3.2; CI= 1.3-8.0; $P<0.05$). The second peak, at week 9, was mainly due to idiopathic peritonitis (Figure 1). Idiopathic peritonitis occurred on 66,7% of the studied cohorts. There was no significant influence of the production system on mortality due to idiopathic peritonitis. However, whereas sporadic cases of idiopathic peritonitis occurred in crossbred and beef cohorts, larger outbreaks (0.4 to 2.0% of the calves) occurred in 4 of the 5 dairy cohorts. The third peak was situated at the end of the production cycle and was almost exclusively due to ruminal disorders and enterotoxaemia (Figure 2).

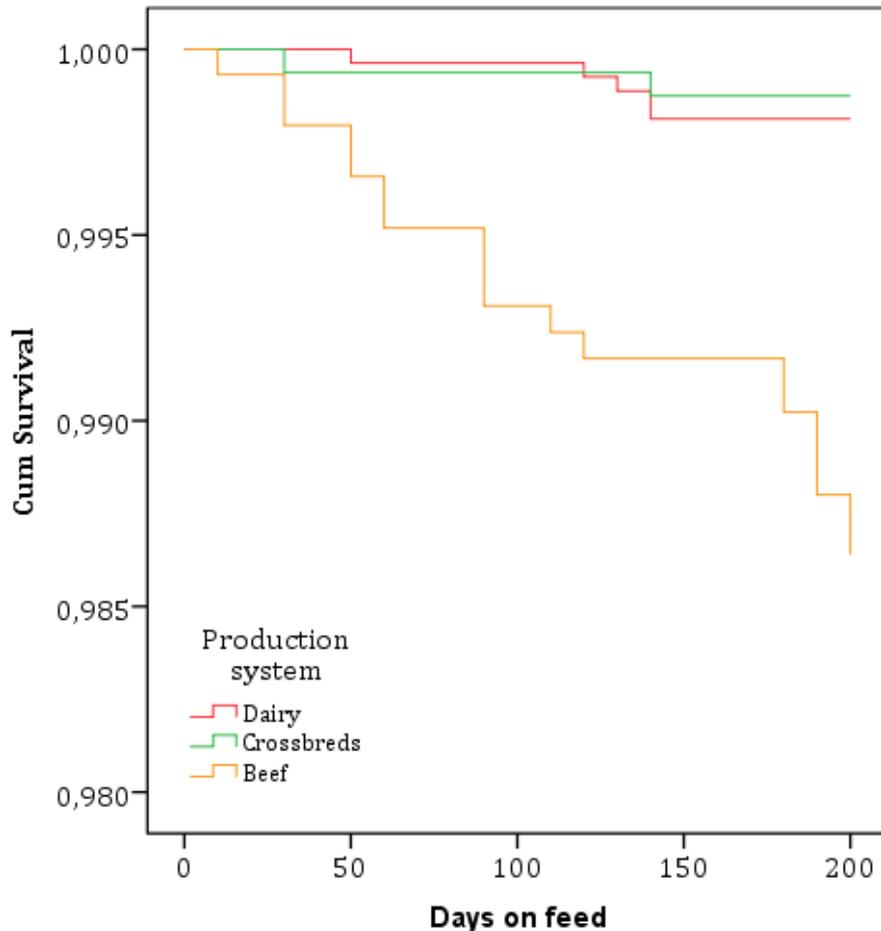


Figure 4. Survival distribution function for enterotoxaemia in white veal calves by production system (15 cohorts, 5853 calves, 2007-2009, Belgium).

Calves housed in the beef production system were much more likely to die from enterotoxaemia than dairy (HR= 7.9; CI= 3.0-20.9; $P<0.01$) or crossbred calves (HR= 11.6; CI= 2.7-49.7; $P<0.01$) (Figure 4). Beef calves were also more likely to die from arthritis, compared to dairy calves (HR= 5.6; CI= 1.0-30.5; $P<0.05$). There was no significant effect of the production system on other causes of mortality. Significant herd effects ($P<0.05$) were noted for total mortality, pneumonia, ruminal disorders, death at arrival and idiopathic peritonitis.

Table 1. Mortality risk (%) and rates (cases per 1000 days at risk) in white veal calves by production system (15 cohorts, 5853 calves, 2007-2009, Belgium)

Cause of mortality	Percentage of cohorts affected	Total (n= 5853)		Dairy (n= 2744)		Crossbreds (n = 1624)		Beef (n= 1485)	
		Mortality risk mean±SD (min-max)	Mortality rate mean±SD (min-max)	Mortality risk mean±SD (min-max)	Mortality rate mean±SD (min-max)	Mortality risk mean±SD (min-max)	Mortality rate mean±SD (min-max)	Mortality risk mean±SD (min-max)	Mortality rate mean±SD (min-max)
Total mortality	100.0	5.3±2.5 (1.8-10.9)	0.27±0.13 (0.09-0.58)	4.9±0.8 (3.9-5.7)	0.25±0.030 (0.22-0.28)	3.5±1.6 (1.8-5.3)	0.18±0.08 (0.09-0.27)	7.5±3.0 (4.8-10.9)	0.38±0.16 (0.25-0.58)
Pneumonia	100.0	1.3±1.1 (0.3-3.5)	0.07±0.06 (0.01-0.18)	0.9±0.5 (0.5-1.8)	0.05±0.03 (0.03-0.09)	0.7±0.3 (0.3-1.2)	0.03±0.02 (0.15-0.06)	2.3±1.5 (0.3-3.5)	0.02±0.08 (0.01-0.18)
Ruminal disorders	86.7	0.7±0.6 (0-2.2)	0.04±0.03 (0-0.11)	0.8±0.6 (0.2-1.6)	0.04±0.03 (0.01-0.08)	0.8±0.8 (0.2-2.2)	0.04±0.04 (0.01-0.11)	0.6±0.6 (0-1.2)	0.03±0.03 (0-0.06)
Enterotoxaemia	66.7	0.5±0.7 (0-2.2)	0.03±0.03 (0-0.11)	0.2±0.2 (0-0.4)	0.01±0.01 (0-0.02)	0.2±0.2 (0-0.5)	0.01±0.01 (0-0.03)	1.3±0.7 (0.5-2.2)	0.06±0.04 (0.03-0.11)
Idiopathic peritonitis	66.7	0.5±0.6 (0-2.1)	0.02±0.03 (0-0.10)	0.8±0.8 (0-2.1)	0.04±0.04 (0-1.10)	0.3±0.3 (0-0.6)	0.01±0.01 (0-0.03)	0.3±0.3 (0-0.8)	0.01±0.02 (0-0.04)
Enteritis	80.0	0.4±0.3 (0-1.3)	0.02±0.02 (0-0.06)	0.3±0.3 (0-0.7)	0.02±0.02 (0-0.04)	0.3±0.3 (0-0.6)	0.01±0.01 (0-0.03)	0.6±0.4 (0.2-1.3)	0.03±0.02 (0.01-0.06)
Death at arrival	20.0	0.3±0.7 (0-2.2)	0.01±0.03 (0-0.11)	<0.1±0.1 (0-0.1)	<0.01±0.00 (0-0.01)	0.4±1.0 (0-2.2)	0.02±0.05 (0-0.11)	0.3±0.7 (0-1.6)	0.02±0.04 (0-0.08)
Arthritis	26.7	0.1±0.3 (0-1.0)	0.01±0.01 (0-0.05)	0.1±0.1 (0-0.2)	<0.01±0.01 (0-0.01)	-	-	0.3±0.4 (0-1.0)	0.01±0.02 (0-0.05)
Abomasal hemorrhage	26.7	0.1±0.1 (0-0.3)	<0.01±0.01 (0-0.02)	0.0±0.1 (0-0.1)	<0.01±0.00 (0-0.01)	0.1±0.1 (0-0.3)	<0.01±0.01 (0-0.02)	0.1±0.1 (0-0.3)	0.01±0.01 (0-0.01)
Congenital heart defect	20.0	0.1±0.2 (0-0.5)	<0.01±0.01 (0-0.03)	0.1±0.2 (0-0.4)	<0.01±0.01 (0-0.02)	0.2±0.2 (0-0.5)	0.01±0.01 (0-0.03)	-	-
Hydranencephalia	20.0	0.1±0.2 (0-0.6)	<0.01±0.01 (0-0.03)	<0.1±0.1 (0-0.1)	<0.01±0.01 (0-0.01)	0.1±0.2 (0-0.5)	0.01±0.01 (0-0.03)	0.1±0.3 (0-0.6)	0.01±0.01 (0-0.03)
Intussusception	20.0	0.1±0.2 (0-0.5)	<0.01±0.01 (0-0.028)	0.0±0.1 (0-0.2)	<0.01±0.01 (0-0.01)	0.1±0.1 (0-0.3)	<0.01±0.01 (0-0.02)	0.1±0.2 (0-0.5)	0.01±0.00 (0-0.03)

Table 1. Mortality risk (%) and rates (cases per 1000 days at risk) in white veal calves by production system (15 cohorts, 5853 calves, 2007-2009, Belgium) (continued)

Cause of mortality	Total (n= 5853)		Dairy (n= 2744)		Crossbreds (n = 1624)		Beef (n= 1485)		
	Percentage of cohorts affected	Mortality risk mean±SD (min-max)	Mortality rate mean±SD (min-max)	Mortality risk mean±SD (min-max)	Mortality rate mean±SD (min-max)	Mortality risk mean±SD (min-max)	Mortality rate mean±SD (min-max)	Mortality risk mean±SD (min-max)	Mortality rate mean±SD (min-max)
Omphalitis	46.7	0.1±0.2 (0-0.6)	0.01±0.01 (0-0.03)	0.2±0.1 (0.2-0.4)	<0.01±0.00 (0.01-0.02)	0.1±0.1 (0-0.3)	<0.01±0.01 (0-0.02)	0.1±0.3 (0-0.6)	0.01±0.01 (0-0.03)
Perforating abomasal ulceration	26.7	0.1±0.2 (0-0.6)	0.01±0.01 (0-0.03)	0.3±0.3 (0-0.6)	0.01±0.02 (0-0.03)	-	-	0.0±0.1 (0.0-0.2)	<0.01±0.01 (0-0.01)
Unknown sudden death	6.7	0.1±0.5 (0-1.8)	0.01±0.03 (0-0.10)	-	-	-	-	0.4±0.8 (0-1.8)	0.02±0.04 (0-0.10)
Iron shock	13.3	<0.1±0.1 (0-0.2)	<0.01±0.00 (0-0.01)	<0.1±0.1 (0-0.2)	<0.01±0.00 (0-0.01)	<0.1±0.1 (0-0.2)	<0.01±0.01 (0-0.01)	-	-
Liver disease	6.7	<0.1±0.1 (0-0.5)	<0.01±0.01 (0-0.024)	<0.1±0.1 (0-0.2)	<0.01±0.00 (0-0.01)	-	-	0.1±0.2 (0-0.5)	0.01±0.01 (0-0.02)
Meningitis	6.7	<0.1±0.1 (0-0.3)	<0.01±0.00 (0-0.02)	-	-	0.1±0.1 (0-0.3)	<0.01±0.01 (0-0.02)	-	-
Mesenteric torsion	6.7	<0.1±0.1 (0-0.3)	<0.01±0.00 (0-0.016)	-	-	-	-	0.1±0.1 (0-0.3)	<0.01±0.01 (0-0.02)

- = no cases

DETAILED NECROPSIES AND ADDITIONAL VIROLOGICAL AND BACTERIOLOGICAL INVESTIGATIONS

From 91 necropsied calves (30%; 91/308) additional samples for bacteriology and virology were taken. Of these calves, 57.1% (52/91) showed lesions of pneumonia, 49.5% (45/91) enteritis (21 catarrhal enteritis and 24 hemorrhagic enteritis), 16.5% (15/91) frothy ruminal bloat, 14.3% (13/91) idiopathic peritonitis, 26.4% (23/91) abomasal ulcerations (3 with perforating ulceration and generalized peritonitis), 19.8% (18/91) fibrinous pleuritis, 5.5% (5/91) pericarditis, 4.4% (4/91) congenital heart defects (3 interventricular septum defects, 1 tetralogy of Fallot), 4.4% (4/91) omphalitis (2 umbilical abscesses, 1 omphalophlebitis with liver abscesses and 1 omphaloarteritis) and 3.2% (3/91) an intussusception. Arthritis, meningitis, hydronephros, abomasal displacement, fistulating hepatitis or orchitis each accounted for one calf. Concurrent enteritis and pneumonia occurred in 22 (24.2%) cases, but the association was not significant. Of the 22 young (< 5 weeks old) calves with enteritis, 50%, 18.1%, 13.6% and 4.6% were *Cryptosporidium parvum*, bovine rotavirus, bovine coronavirus and *Escherichia coli* F5 positive, respectively. *Salmonella spp.* could not be cultured from any of the examined (n= 13) cases with suspicious lesions.

In 92.3% (47/51) of the pneumonia cases a bacterial bronchopneumonia (of which 9 showed pulmonary abscessation) and only in 7.7% (4/51) an acute interstitial pneumonia was found. Chronic pneumonia was associated with fibrinous pleuritis in 17 (33.3%) cases (odds ratio (OR)=9.2; CI= 2.9-29.1; $P<0.01$). Pericarditis was associated with pleuritis (OR= 20.6; CI= 2.1-198.0; $P<0.01$). From pneumonia cases *Mannheimia haemolytica* (19.4%; 7/36), *Pasteurella multocida* (22.2%; 8/36), *E. coli* (30.6%; 11/36), *Trueperella* (formerly *Arcanobacterium*) *pyogenes* (25.0%; 9/36) and *Mycoplasma spp.* (33.3%; 11/33) were isolated. Detection of *T. pyogenes* was associated with pulmonary abscessation (OR= 15.6; CI= 2.2-109.8; $P<0.01$). In 14 cases of acute pneumonia additional virological assays were performed. Of these cases, 35.7% was BRSV PCR positive, whereas BHV-1, PI-3 and BAV-3 were not detected. Overall, 26.0% (19/73) of the examined calves were BVDV PCR positive. A positive BVDV test was associated with chronic pneumonia (OR= 21.6; CI= 5.7-81.9; $P<0.01$) and pleuritis (OR= 4.9; CI= 1.5-16.3; $P<0.01$). Of the 13 cases, classified as idiopathic peritonitis, 8 showed concurrent pneumonia, 4 pleuritis and 1 pericarditis. There were no significant associations

between the presence of peritonitis on the one hand and pneumonia, pleuritis, pericarditis or abomasal ulcerations on the other hand. Bacteriology of abdominal fluid was performed in 3 cases and yielded two times *M. haemolytica* and once *E. coli*.

MORBIDITY

Altogether, 25.4% (896/3519) of the calves developed one or more diseases between arrival and slaughter. The average morbidity risk at the cohort level was 25.0% (SD= 12.9; R=9.6-45.7) and the morbidity rate was 1.7 calves per 1000 days at risk (SD= 1.0; R= 0.6-3.1). In table 2 incidence risk and rates of all individually treated diseases are given by production system. BRD occurred most frequently (56.1% of the initial cases), followed by diarrhea (18.5%), scabies (6.3%, exclusively Belgian Blue), otitis (5.7%) and arthritis (5.5%). The proportion of reoccurrent cases was on average 9.3% (SD=8.8; R= 0-24.3) for BRD, 18.8% (SD= 23.9; R=0-50.0) for necrobacillosis and 0.2% (SD= 0.7; R= 0-2.3) for diarrhea. For other diseases there were no reoccurrent cases. The proportion of relapse cases was 10.2% (SD= 6.1; R= 2.4-20.9) for BRD, 31.3% (SD= 47.3; 0-100.0) for necrobacillosis and 1.3% (SD= 4.0; 0-12.5) for arthritis. Case fatality rate was on average 7.8% (SD= 7.5; R= 0-25.0) for BRD, 25.4% (SD= 33.2; R= 0-100) for arthritis, 6.3% (SD= 12.5; R= 0-50.0) for necrobacillosis, 9.4% (SD= 20.6; 0-66.7) for diarrhea and 42.2% (SD= 51.8; R= 0-100.0) for idiopathic peritonitis.

Table 2. Incidence risk (%) and rates (cases per 1000 days at risk) of individually treated diseases in 3519 white veal calves by production system (10 cohorts, 2007-2009, Belgium)

Disease	Percentage of cohorts affected	Total (n= 3519)		Dairy (n= 1429)		Crossbreds (n = 996)		Beef (n= 1094)	
		Incidence risk mean±SD (min-max)	Incidence rate mean±SD (min-max)	Incidence risk mean±SD (min-max)	Incidence rate mean±SD (min-max)	Incidence risk mean±SD (min-max)	Incidence rate mean±SD (min-max)	Incidence risk mean±SD (min-max)	Incidence rate mean±SD (min-max)
Total morbidity ^a	100	31.0±17.4 (11.2-57.3)	1.66±0.97 (0.57-3.14)	26.1±21.8 (11.2-51.1)	1.45±1.23 (0.57-2.86)	26.6±19.3 (15.4-48.8)	1.39±1.05 (0.78-2.60)	38.0±15.2 (20.8-57.3)	2.03±0.88 (1.05-3.14)
Respiratory disease	100	17.9±9.6 (8.2-33.9)	0.95±0.52 (0.41-1.79)	16.8±9.7 (8.3-27.4)	0.93±0.55 (0.42-1.52)	17.3±14.4 (8.2-33.9)	0.90±0.77 (0.41-1.79)	19.1±8.3 (10.8-28.9)	1.00±0.45 (0.58-1.56)
Diarrhea	100	5.7±3.8 (0-0.5)	0.30±0.20 (0.01-0.61)	3.3±3.9 (0.2-7.7)	0.18±0.22 (0.01-0.42)	6.5±2.3 (4.5-9.1)	0.03±0.13 (0.22-0.47)	7.1±4.5 (1.0-11.4)	0.37±0.24 (0.05-0.61)
Arthritis	100	2.0±2.3 (0.2-7.8)	0.11±0.12 (0.01-0.42)	0.8±0.7 (0.2-1.6)	0.04±0.04 (0.01-0.09)	1.0±0.7 (0.3-1.8)	0.05±0.04 (0.02-0.09)	3.7±2.9 (1.1-7.8)	0.19±0.16 (0.06-0.42)
Scabies	20	1.6±4.4 (0-14.1)	0.09±0.24 (0-0.75)	-	-	-	-	4.1±6.7 (0-14.1)	0.22±0.36 (0-0.75)
Otitis	80	1.3±2.2 (0-2.0)	0.07±0.12 (0-0.40)	2.7±4.1 (0-7.4)	0.15±0.22 (0-0.40)	1.0-0.7 (0.3-1.8)	0.05-0.04 (0.02-0.09)	0.4±0.3 (0-0.6)	0.02±0.01 (0-0.03)
Ruminal drinking	40	0.8-1.7 (0-5.4)	0.04±0.09 (0-0.29)	0.7±1.0 (0-1.8)	0.04±0.05 (0-0.10)	-	-	1.5±2.6 (0-5.4)	0.08±0.14 (0-0.29)
Necrobacillosis	40	0.4±0.6 (0-1.6)	0.02±0.03 (0-0.08)	0.1±0.2 (0-0.4)	0.01±0.01 (0-0.02)	-	-	0.8±0.7 (0-1.6)	0.04±0.03 (0-0.08)
Idiopathic peritonitis	30	0.3±0.8 (0-2.6)	0.02±0.04 (0-0.14)	0.9±1.5 (0-2.6)	0.05±0.08 (0-0.14)	0.1±0.2 (0-0.3)	0.01±0.01 (0-0.02)	0.1±0.2 (0-0.3)	<0.01±0.01 (0-0.02)
Omphalitis	30	0.3±0.7 (0-2.0)	0.02±0.03 (0-0.11)	0.4±0.4 (0-0.7)	0.02±0.02 (0-0.04)	0.7±1.2 (0-2.0)	0.04±0.06 (0-0.11)	-	-
Internal hemorrhage	20	0.2±0.4 (0-1.2)	0.01±0.02 (0-0.07)	0.4±0.7 (0-1.2)	0.02±0.04 (0-0.07)	-	-	0.1±0.1 (0-0.2)	<0.01±0.01 (0-0.01)
Colic	30	0.1±0.2 (0-0.5)	0.01±0.01 (0-0.03)	-	-	-	-	0.3±0.2 (0-0.5)	0.01±0.01 (0-0.03)
Growth retardation	20	0.1±0.4 (0-1.2)	0.01±0.02 (0-0.06)	-	-	-	-	0.3±0.6 (0-1.2)	0.02±0.03 (0-0.06)
Abcessation	10	0.1±0.2 (0-0.5)	<0.01±0.01 (0.01-0.42)	-	-	-	-	0.1±0.3 (0-0.5)	0.01±0.01 (0-0.03)
Neurological symptoms	10	0.1±0.2 (0-0.6)	0.01±0.01 (0-0.03)	-	-	-	-	0.2±0.3 (0-0.6)	0.01±0.02 (0-0.03)
Enterotoxaemia	20	0.05±0.1 (0-0.5)	<0.01±0.01 (0-0.02)	-	-	-	-	0.1±0.2 (0-0.5)	0.01±0.01 (0-0.02)
Trauma	10	0.03±0.1 (0-0.3)	<0.01±0.01 (0-0.02)	-	-	-	-	0.1±0.2 (0-0.3)	<0.01±0.01 (0-0.02)
Eye inflammation	10	0.02±0.1 (0-0.2)	<0.01±<0.01 (0-0.01)	0.1±0.1 (0-0.2)	<0.01±0.01 (0-0.01)	-	-	-	-
Iron shock	10	0.02±0.1 (0-0.2)	<0.01±<0.01 (0-0.01)	0.1±0.1 (0-0.2)	<0.01±0.01 (0-0.01)	-	-	-	-

^aCalves could have more than one disease. Both initial and relapse cases were included. -= no cases; SD= standard deviation

Morbidity peaked in the first 3 weeks after arrival, gradually declined and after week 24 hardly any treatments were initiated (Figure 5). There was no significant difference in total morbidity between the production systems. Diarrhea mainly occurred in the first three weeks after arrival. BRD already occurred immediately after arrival, but the peak incidence occurred at week 3 (Figure 5). Only arthritis was significantly more frequently treated in beef cohorts compared to dairy (OR= 5.3; CI= 1.7-16.8; $P<0.01$) or crossbreds (OR= 3.5; CI= 1.2-10.5; $P<0.01$). Mange was only a problem in Belgian Blue beef calves. The two peaks near the end of the production cycle (day 156 and 166) were due to individual administration of macrocyclic lactones for mange treatment to a large proportion of Belgian Blue calves in cohort 10. There were no other significant associations between production system and diseases. Significant herd effects were detected for total morbidity and all other assessed diseases.

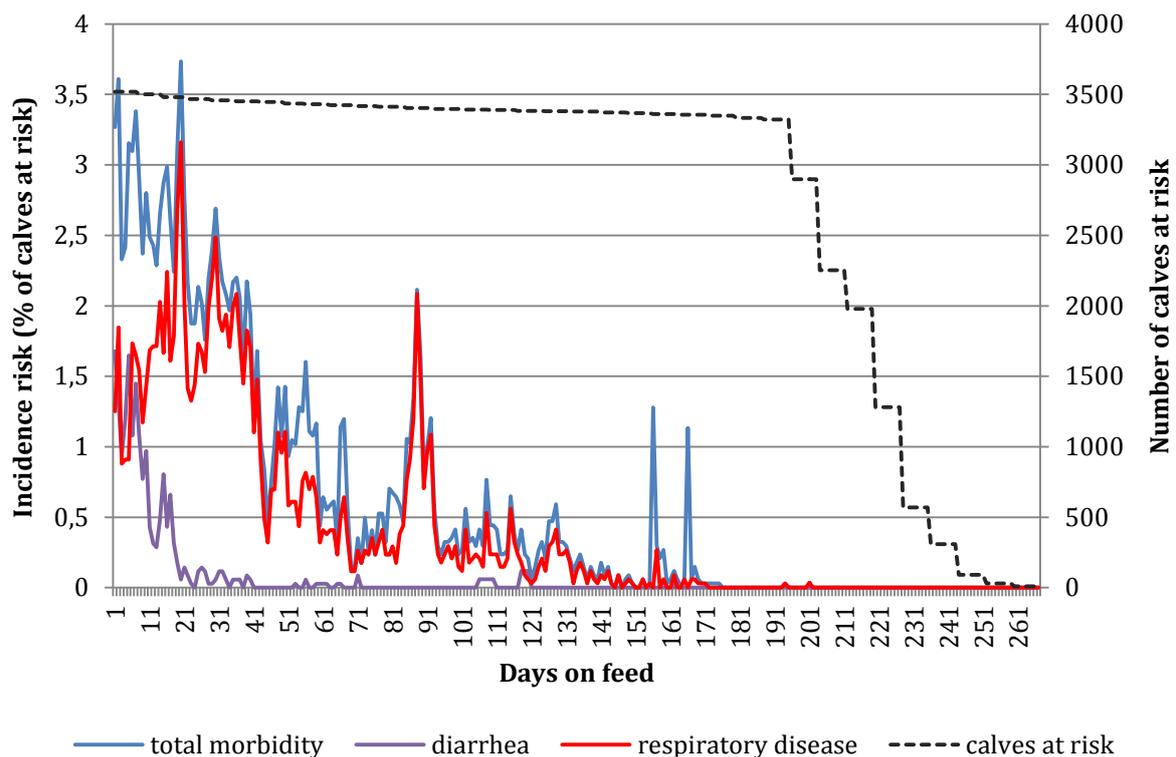


Figure 5. Incidence risk (%) of individually treated diseases according to days on feed in 3519 white veal calves, housed in 10 cohorts in Belgium (2007-2009). A calf was considered morbid on a given day when individually treated with a single or multiple, antimicrobial or non-antimicrobial drug, taking the prolonged effect of the mentioned antimicrobials into account.

DISCUSSION

Random sampling is the method of choice to obtain a representative sample of the population (Thrusfield, 1996). In the present study herds were conveniently selected based upon motivation, as was done in previous studies to guarantee an adequate follow up and to minimize data loss (Olsson et al., 1993; Sargeant et al., 1994a, b; Busato et al., 1997; Donovan et al., 1998; Svensson et al., 2003, 2006a; Bähler et al., 2010). Because the sample size included 5% of the population with more than 90% of the active veterinarians and integrators represented, and because housing and feeding are highly standardized in the Belgian veal industry, the possible selection bias, caused by this selection procedure, is believed to be limited. Because of the convenience selection the sample can only be assumed indicative but not representative for the complete Belgian veal industry at present. The estimation of morbidity was based upon individual treatment by the producer, assuming that treatment rates accurately reflected illness and that increased treatment rates indicated a higher degree of morbidity at that time (Sargeant et al., 1994a,b). Antimicrobial use is however influenced by socioeconomic factors and also the personal attitude of the producer might have influenced the difference in treatment rates between the farms (van der Fels-Klerx et al., 2011). Since easily administrable oral group treatments are frequently used throughout the production cycle, farmers only tended to individually treat calves when severely ill or when only few calves require treatment (Pardon et al., 2012). In that respect, the individual calf treatments do reflect severe individual calf illness as perceived by the producers.

In the present study, the mortality risk (5,3%) was higher than previously reported for white veal calves in Canada (3,7%), the United States (2,5% and 4,2%) and Switzerland (3,0%) (Sargeant et al., 1994b; Stull and McDonough, 1994; Wilson et al., 2000; Bähler et al., 2010). Including beef cohorts in the present study might explain the higher losses compared to studies on dairy veal calves only, since beef calves are more likely to die (Snowder et al., 2005; Maltecca et al., 2006). However, also the mortality risk within the dairy calves was relatively high (4,9%) compared to previous studies. The most likely explanation is probably the longer production cycle (28 weeks) compared to previously studied systems (16-21 weeks), which increased the days at risk. A second explanatory factor might be the housing system. In the older studies, calves were housed in

individual stalls during the complete production cycle. It was postulated that this creates a higher opportunity for individual monitoring and care compared to contemporary group housing (Stull and McDonough, 1994). Also the possibility to control feed uptake in individually housed calves might have been a protective factor, since the mortality risk of digestive diseases was far smaller (19,2%) in individual housing, compared to group housing in Belgium and Switzerland (41,9% and 52,0%, respectively) (Bähler et al., 2010). The exact influence of the housing type on mortality remains unclear, since individual housing is nowadays forbidden and previous comparative studies did not report mortality data (Bokkers and Koene, 2001). Nevertheless, the Swiss study shows that low mortality risks can be achieved in group housing in large pens. However, the fact that in that study, calves were purchased within a day, at a minimum age of three weeks and were housed at low stocking density ($>3,5\text{m}^2/\text{calf}$) most likely also contributed to the lower mortality risk.

High mortality risks (8.2%) have been reported in farms which purchase young calves from different origin (Tyler et al., 1999). Surprisingly, the mortality risk in veal calves was similar to live born calves in dairy replacement herds in Great Britain (5.0%), Norway (4,6%), Sweden (4.0%) and crossbred cow-calf farms in Switzerland (5.0%) and even smaller than reported in large scale dairy calf rearing in Northern America (7,6% and 13,3%) (Sivula et al., 1996b; Busato et al., 1997; Donovan et al., 1998; Svensson et al., 2006c; Ortiz-Pelaez et al., 2008; Gulliksen et al., 2009). Veal producers in Belgium appear to be reasonably able to manage and care for the young, highly stressed calves from multiple origin. However, compared to conventional calf rearing, preventive and metaphylactic antimicrobial drug use plays an important role in this management (Pardon et al., 2012). Whether the current mortality risk can be maintained with less antimicrobial use is an important question for future research.

Compared to North American (5,5%) or Australian bobby calves (0,6%), transport related mortality was low in Belgian veal calves (0.3%), most likely due to shorter transportation times (Staples and Haugse, 1974; Cave et al., 2005). The finding of hydranencephalia in several dummy calves, was associated with the 2007 bluetongue outbreak in Northern Europe and illustrates how close monitoring of veal calves can assist in the detection of calf diseases of global interest (Vercauteren et al., 2008). Diarrhea and related mortality was mainly an issue in the first weeks after arrival,

consistent with the risk period in conventional calf rearing (Sivula et al., 1996b). The incidence rate of diarrhea (0,30 cases per 1000 calf days at risk) was smaller than in Swedish (1.17) and North American (1.50) dairy calves, most likely because calves were also monitored in the neonatal period in the latter studies (Sivula et al., 1996a,b; Svensson et al., 2003,2006c). All major pathogens of the neonatal enteritis complex were found and surprisingly also *E. Coli* F5, suggesting that certain calves were much younger than two weeks old. Although *Salmonella spp.* are historically reported as one of the major causes of mortality in veal calves in Belgium and recent studies still confirmed its presence on Danish veal herds, the bacteria could not be isolated from any of the suspicious cases (van Zijderveld et al., 1982; McDonough et al., 1994; Nielsen et al., 2011). In contrast to conventional dairy calves, diarrhea and respiratory disease occurred simultaneously in the first three weeks after arrival, which is most likely a consequence of commingling (Svensson et al., 2003; Sanderson et al., 2008; Bähler et al., 2010).

Bovine respiratory disease (BRD) was the leading cause of morbidity and mortality. The incidence rate (0,95 cases per 1000 calf days at risk) was similar to dairy calves from Sweden (0.83) or Minnesota (1.00), but smaller than in non-weaned Charolais calves in cow-calf herds (1,89) (Sivula et al., 1996b; Svensson et al., 2003; Assie et al., 2004). Given the large amount of oral group antimicrobial treatments administered for respiratory disease in veal calves, the incidence is probably severely underestimated and a lot more calves is expected to have suffered from respiratory disease than indicated by individual treatment (Thompson et al., 2006). Peak incidences of BRD were reached 2 to 6 weeks after arrival, which is at younger age than conventionally housed dairy heifer calves (10 weeks) (Sivula et al., 1996b). Commingling of calves is a major risk factor for BRD, and the peak incidence of respiratory disease is expected immediately after arrival (Sanderson et al., 2008; Assie et al., 2009b). Metaphylactic treatment at arrival, gradual decline of maternal immunity, incomplete maturation of the immune system and the slowly progressive nature of the dominant pathogens in European veal production, namely *Mycoplasma bovis* and BVDV, might have influenced the occurrence of the peak incidence at the age of 1-1.5 months instead of at arrival (Fulton et al., 2004; Hässig et al., 2007; Arcangioli et al., 2008; Pardon et al., 2011). In contrast to cow-calf herds, where the BRD incidence remains at a higher level (1.0%), hardly any veal calves still require individual treatment after 3 months of age (Sivula et

al., 1996b; Assie et al., 2004). Most likely the similar age and the all-in all-out management of veal calves limit respiratory disease to the first two months after arrival, whereas in conventional herds pathogens can constantly be transferred from older to younger calves. The long tail of the BRD mortality and morbidity curve is explained by a large proportion of chronic BRD cases (reoccurrent and relapse). In addition to previous work, the present study confirms the association of BVDV with chronic pneumonia lesions and pleuritis in white veal calves (Pardon et al., 2011). As in feedlot calves, the synergy between *M. bovis* and BVDV is the cause of chronic, unresponsive pneumonia, often in association with arthritis and otitis (*M. bovis* associated disease) (Haines et al., 2001; Gagea et al., 2006; Arcangioli et al., 2008; Pardon et al., 2011). In this respect, the higher incidence of arthritis and otitis compared to conventional calves is most likely the consequence of the high prevalence of *M. bovis* in white veal cohorts (Svensson et al., 2003). In the present study crossbreds had marked lower mortality due to respiratory disease. This heterosis effect has also been observed in other production systems (Maltecca et al., 2006; Snowden et al., 2005, 2006).

As mentioned earlier, digestive diseases were a more important cause of mortality in the recent studies on group housed calves, compared to an older study on individually housed calves (Sargeant et al., 1994b; Bähler et al., 2010). In group housed veal calves in Switzerland, many more calves died from perforating abomasal ulceration (0.53% vs. 0.11% in the present study) and intestinal torsion (0.4% vs. 0.02%) compared to group housed calves in Belgium (Bähler et al., 2010). In contrast very few calves (0.14%) died from ruminal bloat in Switzerland, whereas ruminal bloat (0.7%) and enterotoxaemia (0.5%) were the most important digestive causes of mortality in Belgium (Bähler et al., 2010). Although both diseases occurred throughout the production cycle, the main risk period was situated near the end of the production round, when feed uptake was at its highest. In contrast to ruminal disorders, enterotoxaemia almost exclusively occurred in Belgian Blue veal calves. The causative agent is *Clostridium perfringens*, but the identity of the toxin and the exact pathogenesis are still unclear (Lebrun et al., 2007; Muylaert et al., 2010). Also Belgian Blue suckler calves are highly susceptible for enterotoxaemia, and it is unclear whether there is a breed predisposition or whether dietary differences between the studied production systems are the cause (Manteca et al., 2001).

Finally, one of the most remarkable causes of mortality in the present study, was idiopathic peritonitis, especially in dairy veal calves. Idiopathic peritonitis emerged only recently in veal calves and the peak incidence at week 9, shortly after the respiratory problems, suggests septicemic spread of bacteria from the lungs to the peritoneum. In one outbreak in Belgium *P. multocida* capsular type F has been isolated from peritoneal fluid in two cases (Catry et al., 2005a). Also, *P. multocida* capsular type B was isolated from outbreaks of pleuritis and peritonitis in intensive dairy calf rearing facilities in New Zealand (McFadden et al., 2011). In the present study no significant association between pneumonia and peritonitis could be demonstrated at necropsy and only *M. haemolytica* and *E. coli* could be isolated from peritoneal fluid. Given these contradictory necropsy results, and the identification of a specific risk period in the present study, more research is necessary to identify the aetiology of idiopathic peritonitis.

CONCLUSIONS

The present study offers a benchmark for morbidity and mortality data in the most common housing system for white veal calves in Europe, based upon the Belgian situation. Respiratory disease was the leading cause of morbidity and mortality. BVDV was associated with chronic pneumonia and pleuritis at necropsy. Calves housed in beef cohorts were at higher risk to die from pneumonia, enterotoxaemia and arthritis. This information can be used to evaluate preventive and therapeutic protocols and can direct producers towards the most profitable strategy with attention for public health and animal welfare.

CONFLICT OF INTEREST STATEMENT

None of the authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

AUTHORS' CONTRIBUTIONS

Conception and design of the study: BP, KDB and PD; Farm visits and follow up: BP, KDB; post-mortem examinations: JC; Data acquisition and statistical analysis: BP, JD, MH; Drafting and critically revising the manuscript: BP, JD, PD. All authors read and approved the final manuscript.

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IMPACT OF RESPIRATORY DISEASE, DIARRHEA, OTITIS AND ARTHRITIS ON MORTALITY AND CARCASS TRAITS IN WHITE VEAL CALVES

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ABSTRACT

Background: Little is known on the effects of common calf diseases on mortality and carcass traits in the white veal industry (special-fed veal), a highly integrated production system, currently criticized for the intensive pro- and metaphylactic use of antimicrobials. The objective of the present study was to determine the impact of bovine respiratory disease (BRD), diarrhea, arthritis and otitis on the economically important parameters of mortality, hot carcass weight (HCW), average daily gain (ADG), carcass quality, fatness degree, meat color and carcass value. For this purpose, a prospective study on 3519 white veal calves, housed in 10 commercial herds, was conducted.

Results: Calves received oral antimicrobial group treatments during 26.1% of the production time on average. Of the calves, 14.8%, 5.3%, 1.5% and 1.6% were treated individually for BRD, diarrhea, arthritis and otitis, respectively and 5.7% died during production. In comparison to non-treated calves, calves treated once for BRD showed a 0.066 kg/day reduction in ADG and 8.2 kg reduction in HCW, had a lower carcass value, a lower fatness degree and an increased mortality risk (odds ratio (OR)= 2.7 (1.9-3.8)). With an increasing number of BRD treatments, these losses increased dramatically. Additionally, calves, treated multiple times for BRD, were more likely to have low carcass quality and an undesirable red meat color at slaughter. Arthritis increased the mortality risk (OR= 3.6 (1.7-7.4)), but reduced HCW only when associated with BRD. Otitis did not affect any of the studied parameters. Diarrhea increased the mortality risk (OR= 3.0 (1.9-4.8)), reduced ADG and HCW by 0.078 kg/day and 8.8 kg, respectively, and decreased carcass quality.

Conclusion: Present results indicate a significant negative impact of BRD, diarrhea and arthritis on economically important parameters in white veal calves, even under the contemporary high levels of antimicrobial coverage. Losses were more pronounced in cases of chronic pneumonia with or without arthritis. Controlling calf health by effective preventive and therapeutic strategies and in particular the prevention of chronic BRD is key for the profitability of veal operations.

BACKGROUND

Several studies in feedlots and dairy operations have shown that calf diseases have an important impact on economic parameters such as mortality, weight gain and carcass traits and that this impact differs according to management and treatment strategies (Virtala et al., 1996; Wittum et al., 1996; Gardner et al., 1999; Fulton et al., 2002a; Snowden et al., 2006; Thompson et al., 2006; Babcock et al., 2009; Reinhardt et al., 2009; Garcia et al., 2010; Stanton et al., 2010; Bach, 2011). Despite the large production scale and high degree of integration in the veal industry, little is known on the effects of common diseases on these economic parameters in contemporary, group housed, white veal calves (special-fed veal) (Pritchard et al., 1981; Postema and Mol, 1984; Catry et al., 2008). The most frequent diseases in white veal operations are bovine respiratory disease (BRD), diarrhea, arthritis and otitis (Pardon et al., 2012b). Whereas most previous studies in different cattle production systems focused on BRD, there is little information on the impact of diarrhea, arthritis and otitis in veal or beef production. Available studies on veal calves either addressed individual housing systems, which are nowadays prohibited in the European Union, or only determined short term disease effects related to the clinical period (Pritchard et al., 1981; Postema and Mol, 1984; Maatje et al., 1993; Stull and McDonough, 1994; Andrighetto et al., 1999; Bokkers and Koene, 2001). In contrast, more recent studies in other cattle production systems, have shown that several of these short term disease effects are less pronounced or no longer meaningful at slaughter (Donovan et al., 1998a; Thompson et al., 2006; Schneider et al., 2009; Bach, 2011). Contemporary veal management implies the intensive use of oral pro- and metaphylactic group antimicrobial treatments, which is highly criticized by the European authorities at present. To what extent calves, that still develop disease under such management, have poorer production results compared to their pen mates is unknown.

Therefore, the objective of the present study was to determine the long term impact of BRD, diarrhea, arthritis and otitis on mortality and carcass traits in white veal calves, raised under contemporary (medical) management.

METHODS

HERDS AND ANIMALS

A prospective cohort study was installed to determine the impact of veal calf diseases on mortality and carcass traits. The study group consisted of 3519 white veal calves, housed in 10 commercial veal farms in Northern Belgium. Participating herds were conveniently selected based upon willingness to cooperate, but independent of any disease history. The sample was stratified on production type (3 dairy (black and red Holstein Friesian (HF)), 4 beef (predominantly Belgian Blue (BB)) and 3 crossbred (HFxBB) herds). Selected herds belonged to six different integrations, including the three largest integrations in Belgium. One all in/all out production cycle per herd (= 1 cohort) was monitored from calf arrival to slaughter, and all calves from that cohort were included in the study. Herds gradually entered the study between January 2008 and October 2009. Calves originated from multiple herds and were transported within 24h from the herd of origin to the veal herds, at the minimum age of 14 days old, after a short stay at a sorting center. Calves were individually housed during the first 6 weeks and thereafter group housed in galvanized pens on slatted floors in compliance with European legislation (Council Directives 91/629/EEC and 97/2/EC). Calves received an all-liquid milk diet, supplemented with solid feed (fibers and concentrates according to European legislation). The milk diet was different between the three production systems: beef calves received predominantly a high quality skimmed milk powder, whereas the skimmed milk diet of dairy and crossbred calves was progressively changed to a lower quality milk powder, based on whey and vegetable proteins, in 8 weeks time. Calves were not vaccinated against any pathogen.

DATA COLLECTION

Calves were individually identified by ear tag, according to Belgian law. Calf entry characteristics (birth date, arrival date, breed, gender) were collected from the Belgian cattle registration system (SANITEL- Animal Health Service-Flanders). The date of mortality and the cause as determined by necropsy were recorded. The used definitions for each cause of death were as previously published (Pardon et al., 2012b). All individual (calf identity, indication and drug) and group treatments, administered by

producer or veterinarian, were recorded daily on written treatment records. Clinical signs on which the producers based their decision to individually treat an animal were the presence of liquid faeces for diarrhea, swollen articulations and/or lameness for arthritis and head tilt for otitis. For respiratory disease producers used mental state, appetite, nasal discharge, cough, rectal temperature ($>39.5^{\circ}\text{C}$) and the presence of tachypnea as criteria. Herds were visited by the primary investigator between 4 and 8 times during the registration period to check compliance with the recording system. Slaughter data (date of slaughter, hot carcass weight (HCW), carcass quality, color and fat classification) were collected at the slaughter houses. The European SEUROP classification system was used to determine carcass quality (18 classes) and scoring was done visually by trained staff. Meat color was determined by spectrophotometry and the European classification system was used (10 classes) (Aporta et al., 1996).

SEROLOGY

Given the diverse etiology of BRD in calves, serology was used to identify the circulating pathogens in each herd. In each herd 25 calves were randomly selected at arrival using the official stable lists. Calves were blood sampled by jugular venipuncture at arrival and 24 weeks later. Serum was collected within 8 hours after sampling and stored at -18°C until analysis. Semi-quantitative indirect ELISA's were used to detect antibodies (IgG1) against bovine respiratory syncytial virus (BRSV), parainfluenza virus type 3 (PI-3), bovine viral diarrhea virus (BVDV) (NS2-3 native protein), bovine adenovirus type 3 (BAV-3), BHV-1 (Respiratory ELISA kit pentakit, Bio-X Diagnostics, Jemelle, Belgium) and *Mycoplasma bovis* (*M. bovis* ELISA kit, Bio-X). Tests were performed according to the manufacturers prescriptions. A seroconversion was considered to have occurred if the signal increases by minimally two orders of magnitude (e.g. 0 to ++; + to +++ or ++ to ++++). Sera from the same calf were tested on the same plate. Antibodies against *Mannheimia haemolytica* (whole cell) were determined at the laboratory of MSD Animal Health (Boxmeer, The Netherlands) with an in-house ELISA. Dilution series of the sera were incubated on plates and bound antibodies were detected after incubation with an anti-bovine serum-peroxidase conjugate (Assie et al., 2009). A four-fold titer increase was considered a seroconversion.

DATA MANAGEMENT AND STATISTICAL ANALYSIS

Mortality, treatment and slaughter data were entered in a relational data base (Access 2007, Microsoft Inc.) and transferred to SAS version 9.3 (SAS Institute Inc., Cary, NC) for descriptive and statistical analysis. A calf was considered a case of a given disease (respiratory disease, diarrhea, otitis and arthritis), when individually treated by the producer or veterinarian for that indication on at least one day. For respiratory disease, each newly installed treatment course more than 14 days after the preceding treatment, was counted as a relapse (Assie et al., 2004; Pardon et al., 2012b). The long acting effect of certain antimicrobial formulations was taken into account by counting one injection as 2 (tilmicosin, amoxicillin, florfenicol, danofloxacin) or 9 days (tulathromycin) of treatment. Binary outcome variables were constructed for mortality, fatness degree, carcass quality and meat color. Fatness degree was split into carcasses with no fat (European class 1) and normal to extremely fat carcasses (European class 2 to 5). All carcasses graded P+ (European class 13) and lower were grouped as insufficient carcass quality and analyzed as such. Also for meat color two classes were created, representing white meat (European class 1 to 6) and meat with an undesirable red color (European class 7 to 10) for veal production.

Hot carcass weight (HCW) (kg), average daily growth (ADG) (kg/day on feed) and carcass value (in €) were continuous outcome variables. HCW was measured at the slaughterhouse at 0.1 kg precise. As experienced by other researchers, the European veal industry does not easily allow weighing calves at arrival and certainly not just before slaughter (Brscic et al., 2012). Therefore, arrival and live slaughter weights needed to be estimated in order to calculate ADG. As arrival weights (kg), average values per breed, available from the veal industry, were used (42 kg for red and black Holstein Friesian (HF), 46 kg for crossbreds (HFxBB) and 56 kg for Belgian Blue (BB) double muscled calves). Live slaughter weights (kg) were estimated by dividing HCW by the average dressing percentage for each breed (55% for HF, 63% for HFxBB and 69% for BB) (Wilson et al., 2000). ADG was calculated by dividing the difference between arrival and live slaughter weight (kg) by the number of days on feed (DOF). DOF (days) was calculated on an individual basis by subtracting the arrival date from the slaughter date. Carcass value was determined for each calf by multiplying HCW by the price per kg (€/kg). The average prices per kg HCW from the years 2008-2009, accounting for

carcass quality and color, were available from the veal industry. Total treatment cost per calf (in €) included the costs related to both group and individual treatments. The cost of oral group treatments was calculated for each herd from the official treatment records, which are under supervision of the federal agency responsible for food chain safety, using the average price (€/kg product) of each drug from the period 2008-2009, available from the veterinary practices that sold these products. The cost of individual treatment for each calf was calculated by multiplying the number of individual treatment days by the average cost of one individual treatment day. An individual treatment day was defined as each day on which an individual calf received one or more individual treatments. The cost of an individual treatment day was calculated by dividing the cost of all individually administered drugs in a herd by the total number of individual treatment days for that herd. Feed cost, labor, housing costs and governmental supports were not taken into account for cost calculation.

The unit of analysis was the individual calf. The effect of the different calf diseases (BRD, diarrhea, arthritis and otitis) on mortality, low fatness degree, red meat color, and low carcass quality was analyzed by multivariable logistic regression. A generalized linear mixed model (PROC GLIMMIX) was used with binomial distribution and logit link function with Wald's statistics for type 3 contrasts. Herd was added as a random factor to account for clustering of calves within a herd. Before building models on the main binary outcome variables (mortality, fatness degree, carcass quality and color), associations between the predictor variables (BRD, diarrhea, otitis, arthritis, age at arrival, breed and gender) were assessed by multivariable logistic regression analysis with herd as random factor. All continuous outcome variables (HCW, ADG and carcass value) were checked for a normal distribution. To determine the effect of calf diseases on these continuous outcome variables a linear mixed model was built (PROC MIXED), with herd included as random effect. In each model, both logistic as linear, besides the calf diseases of interest also breed, age at arrival (days) and gender were evaluated as potential confounders. In the models for HCW and carcass value DOF was included as a continuous independent variable, to account for the production cycle length. In all models, first all predictor variables were tested univariably. All predictors with $P < 0.2$ in the univariable model were withheld for the multivariable model, which was built stepwise backward, gradually excluding non-significant variables. Before entering the predictor variables in the multivariable model Pearson and Spearman's rho correlations

were calculated and when correlation was higher than 0.60, only the most significant variable was retained. For the final models, pairwise comparisons for categorical predictors were made, with Bonferroni adjustment for multiple comparisons. All biologically relevant two-way interactions of significant fixed effects were tested. Significance was set at $P < 0.05$. When necessary to make the models convert, otitis and arthritis were combined into one variable.

RESULTS

Mean size of the studied cohorts was 351.9 calves (Standard deviation (SD)= 121.6; Range (R)= 166-570). Of the 3519 veal calves, 36.8% was black HF, 3.5% red HF, 34.5% BB and 25.1% crossbreds. The majority of the calves was male (91.0%; 3202/3519). The proportion of females was higher in red HF (13.7%; 17/124), BB (9.1%; 111/1215) and crossbreds (16.4%; 145/884) than in black HF (3.4%; 44/1296) ($P < 0.01$). The mean age at arrival was 18 days (SD= 4.8; R= 4-41). Of the calves, 4.1% was of non-Belgian origin and these were exclusively dairy calves. The Belgian calves originated from multiple herds with an average of 1.3 (SD= 0.1; R= 1.2-1.5) delivered calves per herd of origin. The mean time spend in production (DOF) was 192.0 days (SD=33.5; R= 0-281), and was longer in crossbreds (196.5; SD= 31.9) and BB's (196.0; SD= 37.6) than in red (180.7; SD= 47.0) or black HF (186.2; SD= 27.2) ($P < 0.01$). *M. bovis*, BVDV and BAV-3 were the most prevalent pathogens in the studied cohorts (Table 1). Based on standard daily dose methodology, the average treatment incidence was 407.8 daily dosages per 1000 calves at risk. This means that the studied calves received enough oral antimicrobials to treat them for 41,4% of the production cycle length. However, in reality, due to the frequent combination of multiple antimicrobials into one oral treatment, calves received antimicrobials in the milk for on average 26.1% (SD= 10.1; Range (R)= 12.5-40.6) of the time. Further details on drug use are available elsewhere (Pardon et al., 2012a).

In addition to the metaphylactic group treatments, 22.7% (798/3519) of the calves was individually treated for one or more of the studied diseases. Treatment for BRD accounted for 14.8% (522/3519) of the calves, diarrhea for 5.3% (187/3519), arthritis for 1.5% (53/3519) and otitis for 1.6% (57/3519). The average BRD incidence at the cohort level was 17.2%, ranging from 8.2-33.9%. Of the calves, 1.6% (58/3519) and 0.4% (13/3519) relapsed once or more than once for BRD, respectively. The average

day of first treatment for BRD, diarrhea, arthritis and otitis was 40.9 (SD= 36.8), 11.1 (SD= 20.7), 55.9 (SD= 43.9) and 56.6 (SD= 28.6) days after arrival, respectively. Older calves at arrival had less risk to develop diarrhea (odds ratio (OR)= 0.95 per day increase in age; 95% confidence interval (CI)= 0.91-0.98; $P<0.01$), whereas neither age, gender or breed influenced the occurrence of BRD, otitis and arthritis. Calves with diarrhea had higher risks for BRD (OR= 2.8; CI= 2.0-3.9; $P<0.01$) and BRD was associated with increased risks to develop arthritis (OR= 2.2 ; CI= 1.2-4.2; $P<0.05$) and otitis (OR= 2.4; CI= 1.3-4.2; $P<0.01$).

The number of individual treatment days was on average 5.6 days (SD= 5.9) per treated calf, ranging from 1 to 46. The mean cost of individual treatment in these individually treated calves was 50.9 € (SD= 51.1; median= 33.8). The mean cost of the oral group treatments was 7.5 € (SD= 3.8; R= 2.8-13.5). The distribution of all outcome variables (mortality, HCW, ADG, carcass value, meat color, fatness degree and carcass quality) is given by disease in table 2.

Table 1. Seroconversion rate for 7 respiratory pathogens in 10 white veal cohorts, 2008-2009, Belgium

Pathogen	Seroconversion rate (%) (mean±SD)	Range (min.- max.)	Herds affected (%)
Bovine respiratory syncytial virus	8.4±11.4	0-36	70
Parainfluenzavirus type 3	21.2±9.8	12-40	100
Bovine viral diarrhea virus	57.6±27.1	0-84	90
Bovine herpesvirus type 1	3.2±5.9	0-16	30
Bovine adenovirus type 3	50.8±17.1	36-88	100
<i>Mycoplasma bovis</i>	79.6±13.7	56-96	100
<i>Mannheimia haemolytica</i>	32.4±26.4	0-76	80

SD= standard deviation

Table 2. Mortality, average daily growth (ADG) and carcass traits by disease history in 3519 white veal calves from 10 Belgian herds, 2008-2009

Disease	Level	Number of calves ^a	ADG (kg/day) Mean ± SD (min.-max.)	HCW (kg) Mean ± SD (min.-max.)	Low fatness degree ^b % (number)	Red meat color ^c % (number)	Low carcass quality ^d % (number)	Number of calves ^e	Carcass value (€) Mean ± SD (min.-max.)	Mortality, % (number)
Number of treatments for respiratory disease	None	2784	1.13±0.21 (0.17-1.66)	172.6±33.0 (61.0-277.3)	6.1% (160/2629)	14.7% (386/2629)	10.3% (272/2637)	2629	1324.3±568.6 (318.6-2903.4)	4.3% (128/2997)
	1	379	1.06±0.23 (0.33-1.65)	163.3±30.6 (79.8-246.2)	10.6% (39/367)	15.0% (55/367)	7.8% (29/370)	367	1291.2±537.1 (386.0-2577.8)	12.0% (54/451)
	2	39	0.93±0.26 (0.34-1.49)	142.5±38.2 (81.0-251.0)	10.8% (4/37)	27.0% (10/37)	27.0% (10/37)	37	1061.1±584.7 (391.9-2628.1)	20.7% (12/58)
	≥ 3	8	0.77±0.24 (0.46-1.23)	137.6±36.5 (93.8-189.0)	28.6% (2/5)	14.3% (1/7)	42.9% (3/7)	7	1070.3±581.5 (453.9-1792.1)	38.5% (5/13)
Otitis	No	3161	1.12±0.21 (0.17-1.66)	171.4±33.1 (61.0-277.3)	6.9% (205/2992)	15.0% (448/2992)	10.3% (308/3003)	2992	1322.2±566.4 (318.6-2903.4)	5.6% (194/3462)
	Yes	49	1.10±0.19 (0.71-1.46)	146.8±27.5 (98.9-246.2)	0.0% (0/48)	8.3% (4/48)	12.5% (6/48)	48	963.6±391.7 (525.3-2577.8)	8.8% (5/57)
Diarrhea	No	3062	1.12±0.21 (0.17-1.66)	171.3±33.3 (61.0-277.3)	6.7% (196/2907)	15.1% (440/2907)	10.3% (300/2917)	2907	1318.1±567.6 (318.6-2903.4)	5.0% (168/3332)
	Yes	148	1.02±0.21 (0.29-1.61)	164.5±30.1 (87.7-244.9)	6.8% (9/133)	9.0% (12/133)	10.4% (14/134)	133	1281.1±525.6 (469.0-2658.2)	16.6% (31/187)
Arthritis	No	3172	1.12±0.21 (0.29-1.66)	171.1±33.1 (61.0-277.3)	6.7% (202/3014)	15.0% (452/3014)	10.3% (312/3025)	3014	1315.9±565.5 (318.6-2903.4)	5.4% (187/3466)
	Yes	38	0.94±0.30 (0.17-1.43)	167.2±39.2 (65.9-226.7)	11.5% (3/26)	0.0% (0/26)	7.7% (2/26)	26	1389.3±601.3 (496.0-2460.9)	22.6% (12/53)

HCW= hot carcass weight; SD= standard deviation; ^anumber of calves for which ADG and HCW data are available; ^bEuropean class 1 regarded as too low fatness degree; ^cEuropean class 7 to 10 regarded as undesirable red meat color; ^dEuropean class 13 (P⁺) and lower, ^enumber of calves for which carcass value could be calculated

Overall, 5.7% (199/3519) of the calves died before the end of the production cycle, of which 27.1% (54/199) was classified as pneumonia, 7.5% (15/199) as enteritis and 3.5% (7/199) as arthritis. Other important causes of death were acute ruminal disorders (11.0%), enterotoxaemia (10.0%), idiopathic peritonitis (7.0%), death at arrival (5.0%), omphalitis (2.5%) and perforating abomasal ulceration (2.5%) (10.0% of the calves was not autopsied). No calves died from solely otitis. Of the calves, which died from pneumonia, 66.7% (36/54) had been individually treated for BRD. Fatal cases of enteritis and arthritis were individually treated for the respective disease in 40.0% (6/15) and 71.4% (5/7), respectively. The final model for mortality is shown in table 3. All studied diseases, except for otitis, were associated with a higher mortality risk ($P < 0.01$). The odds of mortality markedly increased with increasing number of BRD treatments ($P < 0.01$). Female calves were less likely to die ($P < 0.03$). A trend for a larger mortality risk in BB calves compared to HF was noticed ($P = 0.10$).

Table 3. Final logistic regression model for mortality in 3519 white veal calves, housed in 10 commercial herds in Belgium, 2008-2009

Variable	Level	β	SD	OR	95% CI		P-value
					Lower bound	Upper bound	
Calf gender	Male (ref)	0	-				
	Female	-0.90	0.41	0.41	0.18	0.90	0.03
Number of treatments for respiratory disease	None (ref)	0	-				<0.001
	1	0.99	0.18	2.70	1.90	3.83	<0.001
	2	1.68	0.37	5.35	2.60	11.00	<0.001
	≥ 3	2.41	0.60	11.1	3.39	37.04	<0.001
Diarrhea	No (ref)	0	-				
	Yes	1.11	0.23	3.03	1.93	4.76	<0.001
Arthritis	No (ref)	0	-				
	Yes	1.27	0.37	3.56	1.71	7.41	<0.001

HCW was available for 3210 calves. The remaining calves died during production ($n=199$) or were live exported for slaughter abroad ($n= 309$). The mean HCW was 171.0 kg (SD= 33.2) ranging from 61.0 to 277.3 kg (red HF= 148.9 ± 20.1 ; Black HF= 152.0 ± 24.1 ; Crossbreds= 176.1 ± 27.7 ; BB= 194.1 ± 32.1). Of the variation in HCW, 48% was situated at herd level and 52% at the individual calf level. In a first model all diseases were added separately, not taking the number of BRD treatments into account (Table 4). Breed, gender and DOF were significantly associated with HCW. The interaction of breed with DOF was significant ($P < 0.01$), meaning that next to a breed

effect also the longer production cycle length in BB calves compared to the other breeds has led to heavier carcasses. For example: each day BB calves stayed longer on the farm than the departure date of the first calves (HF's, day 174), HCW increased by 0.7 kg more compared to black HF's. Treatment for BRD and diarrhea ($P<0.01$) were associated with marked weight loss, respectively 25.4 kg and 9.6 kg, compared to untreated calves. In this model the interactions between arthritis and BRD ($P<0.01$) and arthritis and otitis ($P<0.05$) were significant. HCW of arthritis cases only differed significantly from non-treated calves when also treated for BRD. The second interaction was situated in the fact that only arthritis cases, which did not have otitis, differed significantly from the non-treated group. Otitis as such did not cause a reduced slaughter weight. The three way interaction between BRD, arthritis and otitis did not converge. The final model as shown in table 4 explained 31.9% and 7.0% of the variation in HCW at calf and herd level, respectively.

A second model was made in order to assess the effect of the number of BRD treatments on HCW, using a combining variable for otitis and arthritis to make the model converge. HCW decreased severely with increasing number of BRD treatments, namely with on average 8.1 kg, 22.0 kg and 37.2 kg in calves treated once, twice or three and more times, respectively ($P<0.01$) (Table 5). This model showed a slightly better fit and explained 33.8% and 7.4% of the variation at calf and herd level, respectively. Mean ADG was 1.12 kg/day (SD= 0.21), ranging from 0.17 to 1.66. For ADG the herd effect was not significant and regression models without herd as a random factor showed a better fit. The same main effects and interactions as for HCW were noted in the ADG model (model not shown). The model with the number of BRD treatments and the combining variable for otitis and arthritis showed again a slightly better fit (Table 6).

Table 4. Final linear mixed model with pairwise comparisons for hot carcass weight (HCW) (kg) in 3210 white veal calves housed in 10 herds in Belgium, 2008-2009

Variable	Level	Reference	β	SD	P-value
Breed					< 0.001
	Red HF	Black HF	-3.4	2.7	1.0
	Crossbreds	Black HF	4.2	1.8	0.10
	BB	Black HF	9.6	2.2	<0.001
	Crossbreds	Red HF	7.7	2.7	0.02
	BB	Red HF	13.1	2.9	<0.001
	Crossbreds	BB	5.4	1.6	<0.01
DOF (days)					0.93
DOF x Breed					<0.001
	Black HF	BB	-0.7	0.1	<0.001
	Red HF	BB	-0.7	0.2	<0.01
	Crossbreds	BB	-0.5	0.1	<0.001
Calf gender	Female	Male	-10.0	1.6	<0.001
Diarrhea	Yes	No	-9.6	2.0	<0.001
BRD	Yes	No	-25.4	5.2	<0.001
Arthritis	Yes	No	-1.6	8.7	0.85
Otitis	Yes	No	15.8	9.6	0.10
BRD x ART					<0.01
	No BRD/ART	No BRD/No ART	14.3	10.5	1.0
	BRD/No ART	No BRD/No ART	-9.5	1.3	<0.001
	BRD/ART	No BRD/No ART	-27.0	9.7	0.03
	BRD/No ART	No BRD/ART	-24.0	10.6	0.14
	BRD/ART	No BRD/ART	-41.4	10.4	<0.001
	BRD/ART	BRD/No ART	-17.6	9.7	0.42
OTI x ART					0.01
	No OTI/ ART	No OTI/No ART	-25.3	5.3	<0.001
	OTI/No ART	No OTI/No ART	-7.9	3.5	0.14
	OTI/ART	No OTI/No ART	14.2	17.2	1.0
	OTI/No ART	No OTI/ART	17.4	6.3	0.03
	OTI/ART	No OTI/ART	39.5	19.0	0.22
	OTI/ART	OTI/No ART	22.1	17.5	1.0

BB= Belgian Blue; HF= Holstein Friesian; DOF= days on feed; BRD= bovine respiratory disease; ART= arthritis; OTI= otitis; x= interaction

Table 5. Final linear mixed model with pairwise comparisons for the effect of the number of treatments for respiratory disease and other diseases on hot carcass weight (HCW) (kg) in 3210 white veal calves housed in 10 herds in Belgium, 2008-2009

Variable	Level	Reference	β	SD	P-value
Breed					<0.001
	Red HF	Black HF	-3.6	2.7	1.0
	Crossbreds	Black HF	4.0	1.8	0.13
	BB	Black HF	9.6	2.2	< 0.001
	Crossbreds	Red HF	7.6	2.7	0.02
	BB	Red HF	13.2	2.9	< 0.001
	Crossbreds	BB	5.6	1.6	<0.01
DOF (days)					0.71
DOF x Breed					<0.001
	Red HF	Black HF	0.0	0.2	0.95
	Crossbreds	Black HF	0.2	0.1	0.10
	BB	Black HF	0.7	0.1	< 0.001
Calf gender	Female	Male	-10.0	1.6	<0.001
Diarrhea	Yes	No	-9.1	-2.0	< 0.001
ART and/or OTI	Yes	No	-9.6	2.6	< 0.001
BRD					< 0.001
	1 treatment	No BRD	-8.1	1.3	<0.001
	2 treatments	No BRD	-22.0	3.8	<0.001
	≥ 3 treatments	No BRD	-37.2	8.3	< 0.001
	2 treatments	1 treatment	-13.9	3.9	< 0.01
	≥ 3 treatments	1 treatment	-29.1	8.4	<0.01
	≥ 3 treatments	2 treatments	-15.2	9.1	0.58

BB= Belgian Blue; HF= Holstein Friesian; DOF= days on feed; BRD= bovine respiratory disease; ART and/or OTI= combining variable for calves treated for arthritis (ART) and/or otitis (OTI)

Table 6. Final linear mixed model for the effect of the number of treatments for respiratory disease and other diseases on average daily gain (ADG) (kg) in 3210 white veal calves from 10 veal herds in Belgium, 2008-2009

Variable	Level	Reference	β	SD	P-value
Breed					<0.001
	Red HF	Black HF	-0.065	0.02	<0.01
	Crossbreds	Black HF	-0.040	0.01	<0.001
	BB	Black HF	-0.160	0.01	<0.001
	Crossbreds	Red HF	0.025	0.02	1.0
	BB	Red HF	-0.095	0.02	<0.001
	Crossbreds	BB	-0.120	0.01	<0.001
Calf gender	Female	Male	-0.084	0.01	<0.001
Diarrhea	Yes	No	-0.078	0.02	<0.001
ART and/or OTI	Yes	No	-0.070	0.02	<0.01
BRD					<0.001
	1 treatment	No BRD	-0.066	0.01	<0.001
	2 treatments	No BRD	-0.172	0.03	<0.001
	≥ 3 treatments	No BRD	-0.347	0.07	<0.001
	2 treatments	1 treatment	-0.106	0.03	<0.01
	≥ 3 treatments	1 treatment	-0.281	0.07	<0.001
	≥ 3 treatments	2 treatments	-0.175	0.08	0.13

BB= Belgian Blue; HF= Holstein Friesian; BRD= bovine respiratory disease; ART and/or OTI= combining variable for calves treated for arthritis (ART) and/or otitis (OTI)

The risk for an undesirable red meat color was larger in calves, which relapsed for BRD (≥ 2 treatments) (OR= 2.6; CI=1.0-6.6; $P < 0.05$) and increased with increasing age (OR= 1.04 per day increase in age; CI= 1.01-1.06; $P < 0.01$). On the contrary, the combining variable of arthritis and otitis was associated with a lower risk (OR= 0.2; CI= 0.1-0.7; $P < 0.01$). Female calves trended to have a higher risk for red meat color (OR= 1.4; CI= 1.0-2.1; $P = 0.09$). Fatness degree was only affected by BRD, with calves treated once (OR= 2.5; CI= 1.5-4.2; $P < 0.01$) and relapse cases (OR= 3.9; CI= 1.0-15.4; $P = 0.05$) being more likely to have carcasses with low fat content. Calves which were individually treated for diarrhea (OR= 2.5; CI= 1.2-5.4; $P < 0.05$) or relapsed for BRD (two treatments vs. untreated (OR= 10.9; CI= 3.1-38.5; $P < 0.01$) and three or more treatments vs. untreated (OR= 50.0; CI= 3.6-333.3; $P < 0.01$)) had higher odds for low carcass quality (SEUROP score P+ or lower). Also, BB calves had lower odds (OR= 0.07; CI= 0.01-0.42; $P < 0.01$) for low carcass quality compared to red HF.

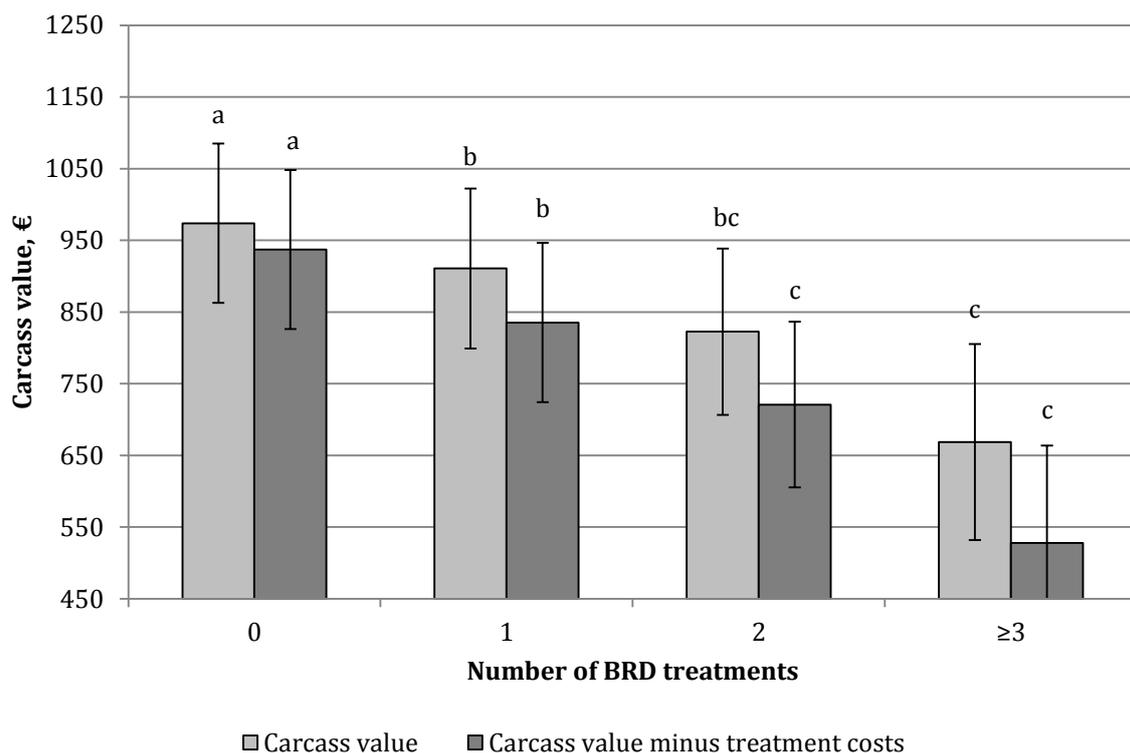


Figure 1. Least square means for actual gross income in white veal calves represented by carcass value alone or by carcass value diminished by group and individual treatment costs, according to the number of administered bovine respiratory disease (BRD) treatments. ^{a-c} Least square means with different letters are statistically different within a series ($P < 0.05$). Bars represent standard deviation.

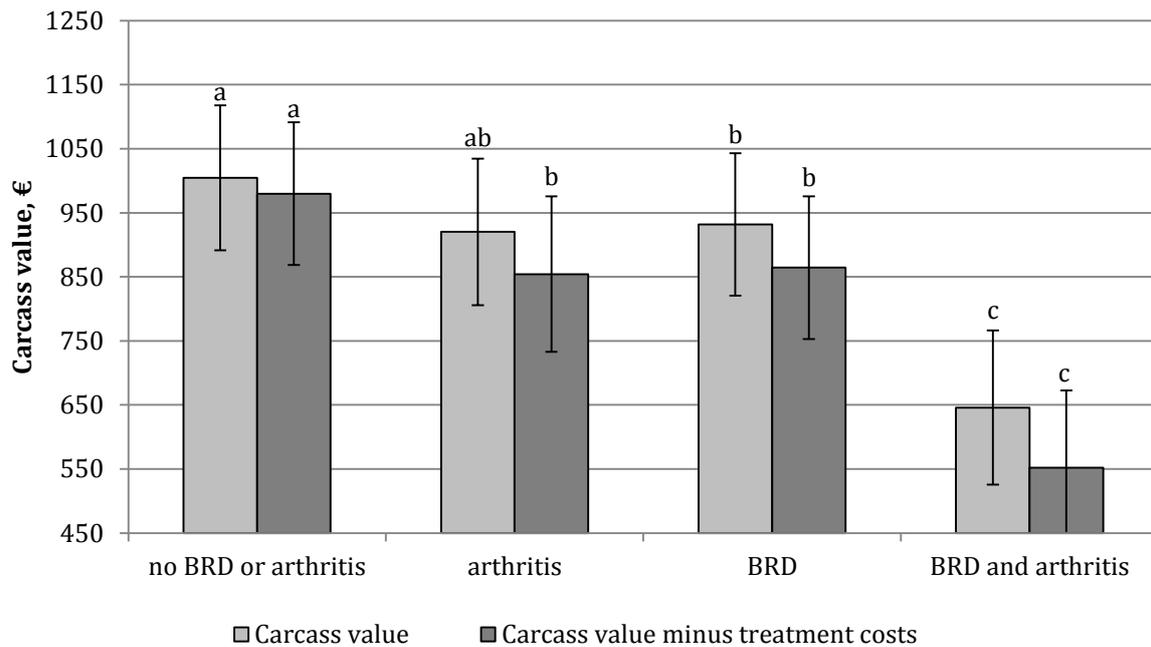


Figure 2. Least square means for actual gross income in white veal calves represented by carcass value alone or by carcass value diminished by group and individual treatment costs, according to individual treatment for bovine respiratory disease (BRD) and arthritis. ^{a-c} Least square means with different letters are statistically different within a series ($P < 0.05$). Bars represent standard deviation.

For carcass value most of the variation (77.6%) was situated at the herd level, likely due to the clustering of breed within herd and the impact of nutrition on the obtained carcass weights. Herd ($P < 0.01$), gender ($P < 0.01$), breed ($P < 0.01$), the interaction of breed with DOF ($P < 0.01$), diarrhea ($P < 0.01$), the combining variable of arthritis and otitis ($P < 0.01$) and the number of BRD treatments ($P < 0.01$) all remained significantly associated with carcass value and with carcass value reduced by the treatment costs in the multivariable models. The final model for carcass value explained 33.5% and 7.9% of the variation at herd and calf level, respectively. Carcass value decreased markedly with increasing number of BRD treatments and more severely when accounting for treatment costs (Figure 1). Arthritis only significantly affected carcass value when occurring together with BRD (Figure 2). When accounting for treatment costs, carcass value of arthritis cases was significantly lower compared to animals that did not develop BRD or arthritis (Figure 2). Also diarrhea resulted in a reduction of 90.2€ (SD= 18.7) and 119.7€ (SD= 18.9) in carcass value and carcass value diminished by treatment costs, respectively.

DISCUSSION

The present study aimed at determining the long term effect of different calf diseases on important economic parameters in white veal production. Besides diseases, many other factors such as nutrition, breed, gender and arrival/birth weight influence growth in calves (Donovan et al., 1998a, b; Galyean et al., 1999). Nutrition explains the greatest proportion of the variation in HCW (Galyean et al., 1999; Bateman et al., 2012). The nutrition effect and the systematic oral antimicrobial group use, which has been shown to have a growth promoter effect in calves, could not be included in the present study since they were colinear with the herd variable and in the case of nutrition also strongly correlated with breed (Cusack, 2004; Berge et al., 2005; Rérat et al., 2011). Despite the absence of nutrition and antimicrobial use as predictors, the final models (including breed and gender) explained a substantial proportion of the variance at calf and herd level, indicating a significant influence of calf health on veal calf performance.

There is no golden standard for BRD diagnosis and previous studies either used producer or veterinarian based clinical diagnosis or lung inspection at slaughter. Neither method is perfect, but lung inspection at slaughter revealed a greater proportion of calves with pulmonary lesions than individually treated (subclinical disease) (Reinhardt et al., 2009; White and Renter, 2009). Yet, in general, both clinical BRD diagnosis as well as lung lesion scoring have been associated with decreased ADG and HCW in cattle (Postema and Mol, 1984; Virtala et al., 1996; Wittum et al., 1996; Donovan et al., 1998b; Gardner et al., 1999; Fulton et al., 2002b; Thompson et al., 2006; Cusack et al., 2007; Step et al., 2008; Schneider et al., 2009; Stanton et al., 2010). In the present study, as in most studies on BRD, producer based diagnosis has been used, because this approach is closest to realistic on farm procedures and therefore better interpretable by the industry itself (Martin et al., 1990; Virtala et al., 1996; Fulton et al., 2002a; Snowden et al., 2005; Thompson et al., 2006; Cusack et al., 2007; Garcia et al., 2010). As experienced by other researchers, the contemporary frequent pro- and metaphylactic oral antimicrobial group treatments interfere with recognition of individual disease (Brscic et al., 2012). For these reasons BRD incidence is likely underestimated in the present study, despite that BRD incidence was in line with the observed incidence in feedlots (17.0% on average, ranging from 4.6-43.8%) and even higher than reported in veal calves in the Netherlands, Italy and France (<7%) (Snowden et al., 2006; Brscic et al.,

2012). Therefore, the results and associations documented in the present study should be interpreted as representing animals with obvious clinical symptoms, with onset of BRD before the installment of the metaphylactic group treatment or calves non-responding to oral group treatment. The overall economic loss due to BRD is likely greater than demonstrated in the present study.

In the present study, veal calves treated once for BRD had a markedly increased mortality risk and lost approximately 8.1 kg carcass weight, which is similar to feedlot cattle (Gardner et al., 1999; Schneider et al., 2009). However, the relative loss in carcass weight is higher in white veal calves compared to feedlot cattle (4.9% vs. 2.3%) and because of the higher prices for veal meat has a greater economic significance (Gardner et al., 1999). BRD has been associated with significant weight loss in the 3 weeks after clinical disease both in feedlots (-0.370 kg/day) as in veal calves (-0.070 kg/day to -0.280 kg/day depending on the installed treatment) (Catry et al., 2008; Schneider et al., 2009). When analyzed over the complete production cycle the loss in ADG is less pronounced (e.g. -0.070 kg/day in feedlots), signifying that after the period of clinical BRD shortly after arrival, subsequent compensatory weight gain occurred in treated animals (Schneider et al., 2009). The fact that BRD cases have a higher eating frequency after the risk period than healthy calves, supports this observation (Buhman et al., 2000). Thompson et al. (2006) reported that BRD associated weight loss was fully compensated at slaughter in South African feedlots, but most other studies on feedlots still found a significant reduction in ADG (-0.040 kg/day to -0.076 kg/day) at slaughter as was the case in the present study (-0.066 kg/day) (Wittum et al., 1996; Gardner et al., 1999; Schneider et al., 2009). This weight loss has been attributed to reduced feed intake due to anorexia and depression in BRD cases and to the increased protein and caloric cost of a febrile response and (chronic) inflammation (Corbeil and Gogolewski, 1985; Blum et al., 1996; Sowell et al., 1999; Buhman et al., 2000; Barnes et al., 2002).

As in feedlots and dairy calves, also in veal calves the reduction in HCW, ADG and carcass value became more pronounced as the number of BRD treatments increased (Van Donkersgoed et al., 1993; Virtala et al., 1996; Gardner et al., 1999; Reinhardt et al., 2009; Schneider et al., 2009). In feedlots chronic unresponsive pneumonia has been associated with *Mycoplasma bovis* and bovine viral diarrhoea virus (Shahriar et al., 2002; Gagea et al., 2006a). Both pathogens were highly prevalent in the studied herds as documented

previously in white veal calves (Arcangioli et al., 2008; Pardon et al., 2011). In addition to pneumonia *M. bovis* causes arthritis and otitis media (*M. bovis* associated disease (MbAD)) (Gagea et al., 2006a,b). Otitis media was not associated with decreased growth nor mortality, as was the case in a dairy heifer raising facility with high incidence of *M. bovis* (Stanton et al., 2010). Despite the high incidence of MbAD in feedlots the effects of arthritis on performance have not been specifically reported. In the present study calves with concurrent arthritis and BRD showed extensive weight loss and a decrease in carcass value similar to chronic BRD cases, whereas calves with only arthritis did not have a significant lower HCW or carcass value. Most likely in the latter calves the arthritis was of traumatic origin and healed after treatment, whereas it was associated with chronic *M. bovis* infection in the calves with concurrent BRD. In calves with chronic arthritis feed uptake is likely further reduced, since the painful joints make them reluctant to move to the drinking trough to eat. As in feedlot calves, chronic BRD also had a significant effect on carcass quality and fatness degree (Gardner et al., 1999; Garcia et al., 2010).

Meat color is an important marketing parameter, which greatly determines carcass value in white veal calves (Sans and De Fontguyon, 2009). The white color of veal meat is obtained by reducing iron uptake, resulting in lower hemoglobin (Hb) and myoglobin levels. However, meat color is affected by many more factors, since Hb only accounted for 29% of the variation in visual color score (Wilson et al., 1995). In the present study, it was shown that chronic BRD results in an increased probability of undesirable red meat at slaughter. A possible explanation, next to the fact that chronic stress causes dark, firm and dry meat, might be that chronic BRD cases, which often suffer from ruminal drinking as well, are more frequently switched to an alternative, more iron rich, concentrate diet (Stocker and Rusch, 1999). Other factors associated with red meat were older age at arrival and female gender. Both effects are not straightforward to explain, but possibly they are related to age and gender differences in iron metabolism as demonstrated in humans and rats (Hahn et al., 2006; Thulluri et al., 2012). In the present study we were obliged to use a combining variable for otitis and arthritis to make the model for meat color convert. To fully understand the influence of BRD, otitis and arthritis on meat color, a dataset large enough to test the interaction between these diseases is necessary and present results on arthritis and otitis should be interpreted with care as to what concerns meat color.

In contrast to BRD few studies have addressed the effects of neonatal calf diarrhea on survival and long term performance. Diarrhea increased the mortality risk in white veal calves, as has been observed in conventional dairy calves in the first 180 days of life (Gulliksen et al., 2009b). In veal calves diarrhea has been associated with significant weight loss in the clinical period (Postema and Mol, 1984). Additionally the present study shows a significant long term effect of diarrhea on HCW, ADG (-0.078 kg/day) and carcass value in veal calves, similar to effects observed in large scale dairy calf rearing (-0.051 kg/day) in the same age period (Donovan et al., 1998b). Whereas Virtala et al. (1996) reported full compensation of neonatal diarrhea associated weight loss at the age of 3 months in small scale dairy calf rearing, the present results and the study of Donovan et al. (1998b) demonstrate that weight loss due to neonatal diarrhea is not fully compensated at the age of 6 months in veal calves and in large scale dairy calf rearing, respectively. It is important to notice that diarrhea also influenced carcass grading, causing additional losses. Despite the fact that calves which had developed diarrhea were predisposed for BRD, as was seen in other studies, the interaction between BRD and diarrhea was not significant in any model, suggesting that both diseases independently significantly affected HCW (Svensson et al., 2006; Gulliksen et al., 2009a).

At present, the high levels of antimicrobial use (especially oral antimicrobial group treatments) and resistance in the veal industry are of great public concern (Catry et al., 2005; Di Labio et al., 2007; Graveland et al., 2010; Cook et al., 2011; Graveland et al., 2011; van Cleef et al., 2011; Pardon et al., 2012a). The present study shows that despite numerous antimicrobial group treatments for BRD and diarrhea, significant production loss still occurred in veal calves, which required additional individual treatment. One reason might be the enormous infection pressure, inherent to the production system, which is unable to be completely overcome by antimicrobial treatment alone. Additional reasons might be the timing of the metaphylactic treatment, the use of antimicrobials for which pathogenic bacteria are resistant, underdosing or insufficiently long individual treatment courses resulting in relapse or persistent subclinical pneumonia (Pardon et al., 2011, 2012a,b). Field studies, evaluating different preventive and therapeutic protocols for their ability to reduce antimicrobial use while maintaining or even improving current production results, are necessary to direct the veal industry towards the most sustainable production strategy.

CONCLUSIONS

Even under the high level of antimicrobial coverage in contemporary white veal production, BRD, diarrhea and arthritis increase the mortality risk and have detrimental effects on growth and carcass traits (weight, quality and value), leading to substantial economic loss. Losses were more pronounced in cases of chronic pneumonia with or without arthritis. Controlling calf health by effective preventive and therapeutic strategies and in particular the prevention of chronic BRD is key for the profitability of veal operations.

COMPETING INTERESTS

Mannheimia haemolytica ELISA's were financially supported by MSD Animal health. The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Conception and design of the study: BP, KDB and PD; Farm visits and follow up: BP, KDB; Data management and statistical analysis: BP, JD, MH; Drafting and critically revising the manuscript: BP, JD, PD. All authors read and approved the final manuscript.

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CHAPTER 5

CHARACTERIZATION OF THE INFECTIOUS COMPONENT OF THE BOVINE RESPIRATORY DISEASE COMPLEX IN WHITE VEAL CALVES

PREVALENCE OF RESPIRATORY PATHOGENS IN DISEASED, NON-VACCINATED, ROUTINELY MEDICATED VEAL CALVES

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ABSTRACT

The prevalence of respiratory pathogens in diseased veal calves was determined in 24 respiratory disease outbreaks on 15 herds in Belgium. Bacteria were cultured from nasopharyngeal swabs and seroconversion against viruses and *Mycoplasma bovis* was determined on paired sera. At the individual calf level, *Mycoplasma spp.*, *Mannheimia haemolytica* and *Pasteurella multocida* were retrieved from 70.5%, 21.5% and 26.0% of the swabs, respectively. At the herd level the presence of *M. bovis* could be confirmed on 84.6% of the examined herds. Seroconversion against bovine viral diarrhoea virus (BVDV), parainfluenzavirus type 3, bovine respiratory syncytial virus (BRSV), bovine adenovirus 3, bovine coronavirus and bovine herpesvirus 1 was present on 71.4%, 53.3%, 40.0%, 46.7%, 30.0% and 26.7% of the herds, respectively. At necropsy, *Mycoplasma spp.* could be cultured from 61.9% of pneumonic lungs (n=21) and respectively 60.0% and 20.0% of the tested calves were BVDV (n=20) and BRSV (n=16) PCR positive. These results demonstrate an overall high presence of *M. bovis* and BVDV in both acute and chronic bovine respiratory disease at the herd level, complementary to a high diversity of viruses and (multiresistant) Pasteurellaceae at the individual calf level.

INTRODUCTION

Bovine respiratory disease (BRD) results from a multifactorial interaction between infectious agents, calf immunity and housing conditions. Usually one or a combination of stressors are needed to induce BRD (Cusack et al., 2003). The microbial aetiology includes viruses and bacteria (including Mollicutes) and their relative contribution varies according to geographical region and production system. The etiology is well documented in several production systems such as the North American feedlots, dairy calf rearing and beef cow-calf operations (Allen et al., 1991, 1992; Van Donkersgoed et al., 1993; Ganaba et al., 1995; Haines et al., 2001; Fulton et al., 2000, 2002; Thomas et al., 2002; Shahriar et al., 2002; Härtel et al., 2004; Gagea et al., 2006a,b; Hägglund et al., 2006; Booker et al., 2008; Angen et al., 2009; Gulliksen et al., 2009). On the contrary, few publications have specifically addressed rearing or fattening herds in which very young calves, from different herds of origin, are transported and commingled (ter Laak et al., 1992; Rusvai and Fodor, 1998; Autio et al., 2007; Caswell and Archambault, 2007; Arcangioli et al., 2008; Radaelli et al., 2008).

The white veal industry is specialized in raising young, predominantly male, calves from the dairy industry on a low iron milk replacer diet. Veal production is present in several European and North American countries (Sans and De Fontguyon, 2009). In France, respiratory disease was studied in group housed (50 animals/pen) veal calves on straw, which were vaccinated against bovine respiratory syncytial virus (BRSV) and bovine viral diarrhoea virus (BVDV). *Mycoplasma bovis* was characterized as the dominant pathogen (Arcangioli et al., 2008). Also, in an Italian study, all veal calves were seropositive for *M. bovis* at slaughter (Radaelli et al., 2008). No information on the pathogens involved in BRD outbreaks in the most frequent production system in Europe (non-vaccinated veal calves, 4-8 animals/pen on a grid floor) is currently available. Knowledge of the pathogens involved in the BRD complex is essential for the implementation and evaluation of evidence based preventive and therapeutic protocols. This is of particular concern since recent studies found a high prevalence of antimicrobial resistance on different body sites from healthy veal calves (Catry et al., 2005, 2007b, Di Labio et al., 2007). The objective of the present field study therefore was to determine the prevalence of respiratory pathogens in non-vaccinated, routinely medicated veal calves suffering from clinical BRD.

MATERIALS AND METHODS

ANIMALS AND HOUSING

The study was carried out between September 2007 and January 2009 on white veal farms with an all-in all-out management in Flanders (Northern Belgium). Calves originated from different European countries (predominantly Belgium) and arrived at an average age of 14.8 days (standard deviation (SD)= 4.5), after a passage through a sorting center. The animals were housed in individual boxes on slatted floors during the first 6 weeks. After this period the metal framework of these individual boxes was removed and the animals were group housed (4-8 animals per pen). The diet consisted out of low iron milk replacer, concentrates and fiber rich roughage. The calves were not vaccinated against any pathogens.

STUDY DESIGN

All Flemish veal veterinarians were asked to report herds with a BRD outbreak. The inclusion criterion was a minimum of 10% of the calves with respiratory disease. At that time, ten diseased calves per outbreak were selected for sampling (acute samples: nasopharyngeal swabs and acute sera; convalescent sera taken three weeks later). A calf was considered a case only if at least 4 of the following 6 symptoms were present: fever (> 39.5°C), nasal discharge, spontaneous cough, increased respiratory rate (>45 breaths per minute), depression and anorexia. Calves individually treated within a week prior to the acute sampling time, were not sampled. Veterinarians were also asked to submit dead calves from the studied herds for necropsy. In total 24 outbreaks on 15 (5.2%) of the 287 veal farms in Flanders were included (Belgian cattle registration system data-Animal Health Service Flanders). On these herds in total 240 diseased calves were sampled. Details on number of animals, breed, age at acute sampling and group antimicrobial treatments before sampling are given in Table 1.

MICROBIOLOGICAL ISOLATION

Nasopharyngeal swabs were taken as previously described (Catry et al., 2005, 2008). Samples were transported at 4-7°C and processed within 24 hours at the laboratory (Animal Health Service Flanders). Isolation of bacteria (Pasteurellaceae and *Mycoplasma*

spp.) was performed according to standard protocols, described elsewhere (Catry et al., 2007a, 2008). Briefly, for Pasteurellaceae the swabs were streaked on a Columbia blood agar (Oxoid, Drongen, Belgium) to which 5% sheep blood and 16 µg/mL bacitracin were added, incubated aerobically at 37°C (for *Histophilus somni* 7% CO₂ was added), and further identified according to Quinn et al., (1994). *Mycoplasma spp.* were isolated on two modified pleuropneumonia-like organisms (PPLO) agars (Difco, Bierbeek, Belgium). Per outbreak a selected number of isolates (one per calf, up to three calves per herd) were stored at -18°C. Strains were cultured again afterwards and further identification of *M. bovis* was done by tDNA PCR on a selected number of isolates (Stakenborg et al., 2005). Antimicrobial susceptibility testing was only performed for isolates from outbreaks which occurred while the calves were receiving oral antimicrobials. The Kirby Bauer disk diffusion technique as earlier described (Catry et al., 2007c) was applied for one index isolate of *Mannheimia haemolytica* and *Pasteurella multocida* per herd. Tested antimicrobials included ampicillin, ceftiofur, flumequine, enrofloxacin, florfenicol, lincospectin, oxytetracycline, sulphonamide-trimethoprim, tylosin and tiamulin.

SEROLOGY FOR RESPIRATORY VIRUSES AND *M. BOVIS*

Blood samples were taken from a jugular vein with a vacuum system (Venoject, Terumo, Leuven, Belgium) immediately after nasopharyngeal swabbing (acute serum) and three weeks later (convalescent serum). Serum was collected and stored at -18°C until analysis. A semiquantitative indirect or competition ELISA was performed to detect antibodies against BRSV (BRSV ELISA kit, Bio-X, Jemelle, Belgium), parainfluenzavirus 3 (PI-3) (Parainfluenza 3 ELISA kit), bovine adenovirus 3 (BAV-3) (Adenovirus 3 ELISA kit), bovine coronavirus (BCV) (Coronavirus ELISA kit) and BVDV (SERELISA BVD p80 mono blocking, Synbiotics Europe SAS, Lyon, France). Additionally, serum from 155 calves (all herds except herds 8, 9, 10 and 11) was analysed by ELISA for antibodies against *M. bovis* (*Mycoplasma bovis* ELISA kit, Bio-X, Jemelle, Belgium). Seroconversion was defined as an increase in signal of at least two magnitudes (0 to ++ or + to +++), according to the manufacturers guidelines. Antibodies against the gE antigen of Bovine Herpesvirus 1 (BHV-1) were detected with an indirect ELISA (HerdChek*Anti-IBR-gE, IDEXX Laboratories, Westbrook, USA). For this test seroconversion was interpreted as a change from negative to positive. Serum samples from the same calf were tested on the

same plate. For analysis of seronegativity in the acute/convalescent serum, samples with magnitudes 0 or + were classified as negative and ++ or +++ as positive samples.

NECROPSY

Necropsy was performed according to an in house standard necropsy protocol (Animal Health Service Flanders). Swabs were taken from lung lesions and processed as described above. In addition, PCR analysis for BVDV (Letellier and Kerkhofs, 2003), BHV-1 (Abril et al., 2004) and BRSV (Boxus et al., 2005) was performed on lung tissue or, in some cases, the BVDV test was performed on spleen tissue. Viral isolation for BAV-3 and PI-3 was performed at the Veterinary and Agrochemical Research Centre (CODA-CERVA, Ukkel, Belgium) according to an in house standard protocol. Briefly, 1 cm³ of lung tissue was suspended in sterile phosphate buffered saline and centrifuged (20', 1500g). The suspension was incubated for 1 hour (5% CO₂, 37°C) with monolayer's of bovine kidney cells. Cells were kept in culture for 12 days and frozen afterwards (-20°C). After fixation of the cells with 4% formaldehyde, 100 µl of this solution was added to each well. Presence of virus particles was determined by indirect immunofluorescence using monoclonal anti-BAV-3 (1/200 diluted) and anti-PI-3 antibodies (1/100 diluted).

STATISTICAL ANALYSIS

Factors (week of outbreak/sampling (equals calf age), season of sampling (winter 2007-2008 or winter 2008-2009), month of sampling, previous antimicrobial therapy for respiratory disease and antimicrobial treatment during sampling) potentially influencing the prevalence of the different pathogens in the diseased animals were evaluated by means of logistic regression always correcting for the herd effect (SPSS statistics version 17.0, SPSS inc., Chicago, Illinois, USA). Significance was set at $P < 0.05$.

RESULTS

CLINICAL OBSERVATIONS

The BRD outbreaks at the veal farms typically were of slow progressive nature rather than sudden outbreaks. The first cases usually occurred within one week after arrival on the farm, but the sampling criterion (10% of the animals with clinical BRD) was on average reached at 22.2 (SD: 15.0) days after arrival. Of the outbreaks, 4%, 33%, 29%, 13% and 21% occurred at 1, 2, 3, 4 or more than 5 weeks after arrival, respectively.

Table 1. General information and antimicrobial group treatments in 24 respiratory disease outbreaks in white veal calves

Outbreak ID	Herd ID	Breed	Compartment Size	Herd size	Sampling week (days after arrival)	Approximate age at sampling (days)	Oral antimicrobial group treatments before sampling	
							Arrival routine (number of days)	Curative BRD treatment (days after arrival)
1	1	HF	52	360	7 (43)	57	TS+Col (10)	Dox (16-21) Amox (30-40)
2	2	BB	52	685	1 (6)	20	Otc+Tyl (10)	Dox (40-45) Otc+Tyl (0-10)
3	3	HF	52	555	2 (12)	26	Amox+Col (10)	Col+Flum (5-15) Tyl (8-13)
4	4	BB	46	325	2 (10)	24	Amox+Col (10)	
5	5	HF	52	167	3 (20)	34	Amox+Col (5)	Dox (13-18) Dox+Tyl (35-40)
6	6	HFxBB	52	650	8 (54)	68	Otc+Col (10)	Amox (36-46) Dox+Tyl (35-40)
7	7	HF	52	580	6 (40)	54	Otc+Col (10)	Amox (16-26)
8	8	BB	46	250	7 (46)	60	TS+Col (10)	
9	9	HF	58	5500	2 (13)	27	Flum (5)	
10	9	HF	58	5500	2 (13)	27	Flum (5)	
11	9	HF	58	5500	3 (20)	34	Flum (5)	
12	9	HF	58	5500	3 (20)	34	Flum (5)	
13	9	HF	58	5500	3 (20)	34	Flum (5)	
14	10	HF	46	210	4 (23)	37	Otc+Col (10)	Amox (12-22) Til (20-23)
15	11	HF	58	443	3 (20)	34	Amox+Col (10)	Otc (13-23)
16	12	HFxBB	46	452	9 (58)	72	Col (10)	Dox+Tyl (7-12) Amox (26-36)
17	9	HF	58	5500	2 (13)	27	Flum (5)	
18	9	HF	58	5500	2 (8)	22	Flum (5)	
19	9	HF	58	5500	2 (8)	22	Flum (5)	
20	9	HF	58	5500	2 (8)	22	Flum (5)	
21	9	HF	58	5500	3 (14)	28	Flum (5)	
22	13	BB	50	435	3 (16)	30	Amox+Tyl (7)	Til (7-10)
23	14	HFxBB	46	301	4 (28)	42	Amox+Col (7)	Tyl (17-23)
24	15	BB	46	182	4 (28)	42	Amox+Col (7)	Tyl (17-23)

HF= Holstein Friesian; BB= Belgian Blue; HFxBB= crossbreed; Amox= amoxycillin; Otc= oxytetracycline; TS= trimethoprim-sulphonamide; Til= tilmicosin; Tyl= tylosin; Col= colistin; Flum= Flumequine; Dox= doxycycline

In 13 outbreaks (54%; 11/15 herds) oral group antimicrobial treatments for BRD had already been initiated prior to the sampling time (first BRD group treatment on average 14.2 (SD: 8.2) days after arrival). In 7 (29%) outbreaks (7/15 herds) oral group antimicrobials were still given at the sampling time (Table 1). In all herds, sampling was done at the BRD incidence peak.

BACTERIOLOGY

In the general bacteriological analysis polybacterial results (n=14) or *Proteus spp.* (n=7) overgrowth were considered as non-interpretable and therefore treated as missing values, leaving 219 swabs for analysis. Due to overgrowth *Mycoplasma spp.* cultures of herds 2 and 7 were also considered as missing values, leaving 220 swabs. From 87% of the swabs (190/219) a well-defined culture could be obtained and 75% (179/219) yielded at least one respiratory pathogen. Commensal or contaminating flora was detected in 15% of the swabs and involved *Staphylococcus aureus* (2.3%; 5/219), *Escherichia coli* (8.7%; 19/219), *Pseudomonas aeruginosa* (0.9%; 2/219), *Streptococcus spp.* (2.7%; 6/219) and *Enterobacter spp.* (0.5%; 1/219). The nasopharyngeal prevalence and seroconversion rates of viral and bacterial pathogens at calf, outbreak and herd level are given in Table 2. Of the calves, only 2.7% (6/219) carried both *P. multocida* and *M. haemolytica* in the nose, 22.6% (46/203) *P. multocida* and *Mycoplasma spp.*, 16.2% (33/203) *M. haemolytica* and *Mycoplasma spp.* and 2.5% (5/203) carried all three pathogens. The prevalence of *Mycoplasma spp.* was significantly higher in animals sampled at younger ages in comparison to animals sampled at older ages (>5 weeks after arrival) ($P<0.05$). No other significant risk factors could be identified on the present dataset. In 3 of the 7 outbreaks in which the calves were treated with oral antimicrobials at the sampling time (herds 1, 2 and 15), both *M. haemolytica* and *P. multocida* could be isolated from 1 to 7 calves per outbreak, and in two outbreaks (herd 7 and 14), only *P. multocida* (1 and 2 calves, respectively) could be isolated. Antimicrobial susceptibility testing of these isolates showed resistance to tylosin and oxytetracycline in herd 1, to tylosin in herd 2, to oxytetracycline in herd 7 and to oxytetracycline, ampicillin, flumequine, enrofloxacin, tylosin and tiamulin in herd 15 (systematically the same resistances in *P. multocida* and *M. haemolytica*) (herd 14 not tested).

Table 2. Prevalence of respiratory pathogens in diseased veal calves at calf, outbreak and herd level

Sample	Agent	Calf level	Outbreak level	Mean number of positive calves (range; max: 10)	Herd level
		(n=219)	(n=24)		(n=15)
		Percentage of positive samples (positives/No. sampled)	Percentage of positive outbreaks (positives/No. sampled)		Percentage of positive herds (positives/No. sampled)
	<i>M. haemolytica</i> ^a	21.5 (47/219)	66.7 (16/24)	2 (0-7)	46.7 (7/15)
	<i>P. multocida</i>	26.0 (57/219)	75.0 (18/24)	2 (0-7)	73.3 (12/15)
NPS ^c	<i>H. somni</i>	0 (0/219)	0 (0/24)	0	0 (0/15)
	<i>A. pyogenes</i>	2.7 (6/219)	16.7 (4/24)	0 (0-2)	26.7 (3/15)
	<i>Mycoplasma spp.</i> ^b	70.8 (155/220)	95.4 (21/22)	7 (0-10)	92.3 (12/13)
	<i>M. bovis</i> ^b	-	90.9 (20/22)	-	84.6 (11/13)
	<i>M. bovis</i>	32.9 (51/155)	87.5 (14/16)	3 (0-7)	81.8 (9/11)
	BRSV	4.3 (10/233)	29.2 (7/24)	0 (0-2)	40.0 (6/15)
	PI-3	9.4 (22/233)	54.2 (13/24)	1 (0-5)	53.3 (8/15)
Serum ^d	BAV-3	7.7 (18/233)	37.5 (9/24)	1 (0-4)	46.7 (7/15)
	BHV-1	3.9 (9/233)	16.7 (4/24)	0 (0-6)	26.7 (4/15)
	BCV	5.4 (11/202)	33.3 (7/21)	1 (0-3)	30.0 (4/12)
	BVDV	18.9 (40/212)	73.9 (17/23)	2 (0-4)	71.4 (10/14)

BRSV= bovine respiratory syncytial virus; BVDV= bovine viral diarrhoea virus; PI-3= parainfluenzavirus 3; BHV-1= bovine herpesvirus 1 ; BAV-3= bovine adenovirus 3; BCV= bovine coronavirus.

^a*Mannheimia haemolytica sensu lato*

^bNo species identification at the calf level. Identification (*M. bovis* PCR) only for selected isolates per outbreak.

^cNPS= nasopharyngeal swab

^dSeroconversion rates: 7 calves died before the convalescent serum was taken, leaving 233 paired sera. For *M. bovis*, BCV and BVDV, 155, 202 and 212 paired sera could be analyzed, respectively.

SEROLOGY

Convalescent sera were not available for 7 calves for reasons of death, leaving 233 paired sera. In 40.3% (94/233) of the calves seroconversion for at least one virus was found. Seroconversion rates at calf, outbreak and herd level are given in Table 2. Seroconversion against BCV and BVDV was not tested for herds 2 and 10, and herd 3 respectively. At the outbreak level, on average seroconversion against 2 viruses (range: 0-4) was detected. Only in outbreak 6 no viral component was identified. At the individual calf level multiple viral infections were rare (5.6%; 13/233) and predominantly involved BVDV (9/13) and BAV-3 (9/13). Only three (1.3%) calves seroconverted for three or more viruses. Among the 155 calves for which *M. bovis* serology was available, 32.9% seroconverted for *M. bovis*. Of these 155 calves, 21.3% also seroconverted against at least one virus (BVDV (5.2%), PI-3 (3.9%), BCV (3.9%), BAV-3 (2.6%), BHV-1 (1.9%), and BRSV (1.9%)). The prevalence of seronegatives in the acute serum and the changes in serological status are given in Table 3. Of the calves, 4.3%, 24.0%, 27.9%, 24.9%, 14.2%, 4.3% and 0.4% were seropositive in the acute serum against all 6, 5, 4, 3, 2, 1 or no viruses, respectively.

Table 3. Prevalence of seronegative calves and changes in serological status for respiratory viruses and *Mycoplasma bovis*

Agent*	Percentage of seronegative calves		Percentage of calves seroconverting (number/sampled calves)	Seronegatives in the acute serum that seroconvert (%) (number/sampled calves)
	(No. seronegatives/ No. sampled calves)			
	Acute serum	Convalescent serum		
BRSV	32.6% (76/233)	54.9% (128/233)	4.3% (10/233)	13.2% (10/76)
PI-3	13.3% (31/233)	10.3% (24/233)	9.4% (22/233)	71.0% (22/31)
BAV-3	36.1% (84/233)	41.6% (97/233)	7.7% (18/233)	21.4% (18/84)
BHV-1	77.7% (181/233)	79.8% (186/233)	3.9% (9/233)	5.0% (9/181)
BCV	13.4% (27/202)	21.3% (43/202)	5.4% (11/202)	40.7% (11/27)
BVDV	45.8% (97/212)	30.2% (64/212)	18.9% (40/212)	41.2% (40/97)
<i>M. bovis</i>	67.7% (105/155)	22.6% (35/155)	32.9% (51/155)	51.0% (54/105)

*BRSV: bovine respiratory syncytial virus; PI-3: parainfluenzavirus type 3; BAV-3: bovine adenovirus type 3; BHV-1: bovine herpesvirus-1; BCV: bovine coronavirus; BVDV: bovine viral diarrhea virus

POSTMORTEM EXAMINATION

A total of 21 calves from 8 different herds underwent necropsy. The average age was 62.9 (SD: 24) days, which was on average 29.1 (SD: 26) days after the acute sampling date. Details on the necropsies are given in Table 4. From the lungs *Mycoplasma spp.* (61.9%), *P. multocida* (9.5%), *M. haemolytica* (9.5%) and *Arcanobacterium pyogenes* (28.6%) could be isolated. Of the examined calves (n=20), 60% were BVDV PCR positive on pulmonary or spleen tissue, whereas 20.0% of the examined calves (n=15) were BRSV PCR positive.

Table 4. Necropsy findings, bacteriology and virology in 21 veal calves with respiratory disease

Calf ID	Outbreak ID	Days after arrival	Age (days)	Necropsy findings	Bacteriology (culture)	Virology				
						BRSV (PCR)	BVDV (PCR)*	BHV1 (PCR)	BAV †	PI-3 †
1	1	43	57	Chronic catarrhal bronchopneumonia	<i>Mycoplasma spp.</i> <i>M. haemolytica</i> <i>E. coli</i>	-	+	-	-	-
2	1	52	65	Chronic catarrhal bronchopneumonia	<i>Streptococcus spp.</i> <i>E. coli</i>	-	+	-	-	-
3	3	12	26	Subacute catarrhal bronchopneumonia	<i>A. pyogenes</i>	+	+	-	-	-
4	3	50	64	Chronic catarrhal bronchopneumonia Hydranencephalia	<i>P. aeruginosa</i>	-	+	-	-	-
5	3	50	64	Chronic caseo-necrotic pneumonia Pleuritis	<i>Mycoplasma spp.</i> <i>A. pyogenes</i> <i>Mycoplasma spp.</i>	-	+	-	-	-
6	3	50	64	Chronic catarrhal bronchopneumonia	<i>A. pyogenes</i> <i>P. multocida</i>	-	+	-	-	-
7	4	37	51	Lung oedema + emphysema Peritonitis	<i>E. coli</i>	ND	ND	ND	ND	ND
8	9	45	59	Chronic catarrhal bronchopneumonia	<i>M. bovis</i> <i>M. haemolytica</i> <i>P. aeruginosa</i>	-	+	-	-	-
9	9	69	83	Chronic catarrhal bronchopneumonia	<i>E. coli</i> <i>Proteus spp.</i>	+	-	-	-	-
10	9	88	102	Chronic caseo-necrotic pneumonia Pleuritis	<i>M. bovis</i>	-	+	-	-	-
11	9	92	106	Chronic caseo-necrotic pneumonia Pleuritis	<i>M. bovis</i> <i>A. pyogenes</i> <i>P. multocida</i> <i>S. bovis</i>	-	-	-	-	-
12	15	11	25	Subacute catarrhal bronchopneumonia Pleural effusion	<i>Proteus spp.</i>	+	-	-	-	-
13	15	20	34	Chronic caseo-necrotic pneumonia Pleuritis	<i>Proteus spp.</i>	-	+	-	-	-
14	17	38	52	Chronic catarrhal bronchopneumonia	<i>M. bovis</i> <i>A. pyogenes</i>	-	-	-	-	-
15	17	39	53	Chronic catarrhal bronchopneumonia	<i>M. bovis</i>	-	-	-	-	-
16	17	46	60	Chronic catarrhal bronchopneumonia	<i>M. bovis</i> <i>A. pyogenes</i>	-	-	-	-	-
17	23	8	22	Subacute catarrhal bronchopneumonia Peritonitis	<i>M. bovis</i>	ND	-	ND	ND	ND
18	23	65	79	Chronic catarrhal bronchopneumonia	<i>Lactobacillus spp.</i>	ND	+	ND	ND	ND
19	23	65	79	Chronic broncho-pneumonia + abscess	<i>M. bovis</i> <i>Proteus spp.</i>	ND	+	ND	ND	ND
20	24	98	112	Chronic catarrhal bronchopneumonia Abomasal ulceration	<i>M. bovis</i>	ND	-	ND	ND	ND
21	24	49	63	Chronic caseo-necrotic pneumonia	<i>M. bovis</i> <i>A. pyogenes</i>	ND	+	ND	ND	ND

ND: not determined; +: positive; -: negative; BRSV: bovine respiratory syncytial virus; PI-3: parainfluenzavirus type 3; BAV-3: bovine adenovirus type 3; BHV-1: bovine herpesvirus-1; BVDV: bovine viral diarrhoea virus; * BVDV PCR on lung tissue for all calves, except calves 17 to 21; † determined by virus isolation

DISCUSSION

The objective of the present study was to determine the prevalence of respiratory pathogens in non-vaccinated white veal calves, suffering from respiratory disease. Deep nasopharyngeal swabs were chosen to identify the bacterial component, because this sampling procedure is quick, simple and of minimal invasive nature (Godinho et al., 2007). Most respiratory bacteria are ubiquitous and can be detected in both healthy and ill calves by nasopharyngeal swab and broncho-alveolar lavage (BAL) (Allen et al., 1991; Autio et al., 2007). At the group level a good association between cultures from nasopharyngeal swabs and BAL's has been demonstrated for *M. haemolytica*, *P. multocida* and *M. bovis* (Allen et al., 1991; De Rosa et al., 2000; Godinho et al., 2007).

In the diseased veal calves, the nasal prevalence of *P. multocida* was lower (26.0% vs. 33.6%) and of *M. haemolytica* higher (21.5% vs. 5.9%) than in a previous study on healthy veal calves in Belgium (Catry et al., 2005). In contrast to previous studies in feedlot cattle and similar to another study on veal calves in France, *H. somni* was not isolated (Allen et al., 1992; Haines et al., 2001; Shahriar et al., 2002; Arcangioli et al., 2008). The fact that in 7 herds calves were under oral antimicrobial treatment at the sampling time, might have negatively influenced the detection rate of the Pasteurellaceae. Nevertheless, likely due to the high level of antimicrobial resistance in this type of calf rearing (all tested isolates in these particular herds were also (multi)resistant) or due to insufficient antimicrobial concentrations for elimination in the upper respiratory tract, Pasteurellaceae were still detected in 5 of these 7 herds. The finding of multiresistant isolates in these herds, but moreover the occurrence of clinical BRD during such oral antimicrobial treatments, highlights the potential of therapeutic failure due to multiresistant Pasteurellaceae. Further studies are necessary to confirm the importance of this observation.

In recent years the prevalence of *M. bovis* increased in several central European and American countries, predominantly in high density production systems where calves from multiple origin are commingled (Haines et al., 2001; Shahriar et al., 2002; Gagea et al., 2006b; Arcangioli et al., 2008; Radaeli et al., 2009). On the contrary, in Norwegian, Finnish and Danish dairy calves, *M. bovis* was not found (Autio et al., 2007; Angen et al., 2009; Gulliksen et al., 2009). In 84.6% of the tested herds (n=13) in this study, the presence of *M. bovis* could be confirmed, which is similar to the situation in French veal

calves (88.9%) (Arcangioli et al., 2008). Of the examined calves, 32.9% seroconverted for *M. bovis* during acute BRD. In previous studies between 60 and 100% of the calves seroconverted between arrival and one or two months later, respectively, and at slaughter all calves were seropositive (Arcangioli et al., 2008; Radaelli et al., 2009). In the present study, 32.3% of the calves were already seropositive in the acute sample (3 weeks after arrival), compared to only 2.2% at arrival in the study of Arcangioli et al., (2008). This difference can be explained either by a higher level of maternal antibodies against *M. bovis* in Belgian cattle or, more likely, by seroconversion of several calves in the first two weeks after arrival, before the BRD peak incidence was reached. At necropsy, the typical caseonecrotic pneumonia, associated with *M. bovis* infection, was seen in several cases. *Mycoplasma spp.* were isolated from 61.9% of the necropsied cases and in all calves in which species identification was performed (n=10), *M. bovis* was identified. In North American feedlots *M. bovis* was isolated from 82 to 92% of pneumonic lungs at necropsy (Haines et al., 2001; Shahriar et al., 2002). A possible explanation for the lower prevalence in the present study is the presence of polybacterial overgrowth in several calves, which most likely resulted from a too long time delay between death and necropsy.

When commingling neonatal calves from different herds of origin, maternal immunity will greatly determine their susceptibility towards viruses. Maternal antibodies can persist for months and their decline depends on the amount of antibodies ingested and absorbed and on the infection pressure (Fulton et al., 2004). The commercial antibody ELISA's used in the present study detect IgG1, which is the dominant maternal antibody. Therefore, the results give an impression of the presence of maternal immunity. In the acute serum 81% of the calves was seropositive against three or more viruses and this is reflected in the fact that in none of the outbreaks a single virus caused seroconversion in all sampled calves. The highest percentages of seronegatives in the acute serum were found for BVDV and BHV-1. BVDV was in this study the major cause of viral seroconversion. A synergy between *M. bovis* and BVDV infection has been described in cases of chronic unresponsive respiratory disease and/or arthritis in North American feedlots (Haines et al., 2001; Shahriar et al., 2002; Gagea et al., 2006a). In the present study, the necropsied calves were also predominantly chronic cases and both BVDV and *M. bovis* could frequently be demonstrated. The number of acutely ill calves in which a simultaneous seroconversion for *M. bovis* and BVDV could be demonstrated, was

however limited (5.2%; 8/155). BVDV was also frequently involved in multiple viral infections as described in feedlots and dairy calves (Richer et al., 1988; Fulton et al., 2000).

Seroconversion rates for BHV-1 were low, which was also observed in French fattening bulls and is probably the consequence of national campaigns against BHV-1 in different European countries (Assié et al., 2009). Nevertheless, the occurrence of seroconversion against BHV-1 on 26.7% of the examined herds, remains worrisome and special attention should be given to importing calves from endemic regions. The lower prevalence of PI-3 and BRSV in the present study compared to French veal calves in a previous study, might be explained by the fact that in the French study seroconversion between arrival and 2 months later was determined, instead of related to the clinical period as in the present study (Arcangioli et al., 2008). For BRSV it is well known that maternal antibodies do not protect against infection and can suppress serum responses (Kimman et al., 1987, 1988). Seroconversion of seronegatives in the acute serum is however useful and shows that only a minority (13.2%) of seronegative calves seroconvert. Nevertheless the virus was detected in 20% of the examined calves at necropsy, whereas BHV-1, BAV-3 and PI-3 weren't. The presence of BRSV was confirmed on 40% of the herds, but the possible involvement in BRD on the other herds cannot be excluded, because of the before mentioned reasons. The percentage of calves seroconverting for BAV-3 was lower than in previous studies in similar settings (7.7% vs 13% (Autio et al., 2007) and 19% (Nikunen et al., 2007)). Of the seronegative calves in the acute serum, 40.7% seroconverted against BCV. Although seroconversion does not distinguish between a respiratory or intestinal infection, involvement of BCV in respiratory disease is likely as concurrent faecal and nasal shedding occurred in 38% of infected calves in a previous study (Hasoksuz et al., 2002).

In summary, respiratory disease in white veal calves is of slow progressive nature rather than massive acute outbreaks, likely due to the presence of maternal immunity and the frequently applied metaphylactic antimicrobial therapy. The peak incidence is under such conditions on average reached at week 3 post arrival. At that time, next to a variable viral component in the individual calf, (multi)resistant Pasteurellaceae are prevalent. Overall, *M. bovis* and BVDV appear to play an important role in both the initiation of BRD (acute outbreak) as in lethal chronic cases. Therefore, antimicrobial

therapy should be adjusted to the natural resistances of *M. bovis* and the acquired resistance profile of isolated Pasteurellaceae. As in 40% of the calves a viral component was identified, also vaccination (BRSV, PI-3 and BVDV) or excluding BVDV persistently infected calves from production might be economically beneficial. However, the potential effect of the latter actions on the economic result and the reduction of antimicrobial use in the white veal industry remains to be determined.

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SEROEPIDEMIOLOGY OF RESPIRATORY INFECTIONS IN WHITE VEAL CALVES UNDER ANTIMICROBIAL COVERAGE AND ASSOCIATIONS WITH RESPIRATORY DISEASE AND CARCASS TRAITS

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ABSTRACT

The dynamics and predictive value of antibodies to 7 respiratory pathogens were studied in 467 white veal calves (15 herds). With exception of *Mycoplasma bovis*, maternal antibodies against most pathogens were abundantly present, but 40,1% of the calves had insufficient total immunoglobulin (IgG) levels at arrival. The respiratory disease (BRD) peak in the first 6 weeks after arrival was associated with seroconversion to *M. bovis* (44.2% of the calves) and bovine viral diarrhoea virus (BVDV; 32.0%). Of the calves, 0.6% was persistently infected with BVDV. Calves, seropositive to parainfluenzavirus type 3 and *M. bovis* at arrival, had a decreased and increased risk for BRD, respectively. Low total IgG at arrival tended towards an increased BRD risk. No associations with carcass traits were found. Under the current medical management, the serostatus for respiratory pathogens at arrival was of little practical use to classify veal calves according to BRD risk.

INTRODUCTION

In veal production bovine respiratory disease (BRD) is the leading cause of morbidity and mortality and the main indication for antimicrobial drug use (Pardon et al., 2012a,b). The high levels of antimicrobial use and resistance in the veal industry are currently strongly criticized, because of the potential risk for human health (Catry et al., 2005; Hammerum and Heuer, 2009; Graveland et al., 2010). Vaccination and the classification of calves according to BRD risk, to target metaphylactic therapy at arrival, are two possible ways to reduce antimicrobial use. To support these measures, knowledge on maternal antibody levels, and on the prevalence and spread of respiratory pathogens in white veal calves is essential, but this is hardly available in literature. In feedlots, high antibody titers at arrival to different respiratory pathogens could be associated with a reduced BRD risk (Martin et al., 1998, 1999). Also, calves persistently infected (PI) with bovine viral diarrhoea virus (BVDV), had higher BRD risks and low survival rates (Loneragan et al., 2005). In neonatal beef and dairy calves, failure of passive transfer (FPT) has been associated with increased morbidity and mortality risks (Donovan et al., 1998; Stilwell and Carvalho, 2011). Both the prevalence of FPT and PI calves in the European veal industry and their association with BRD have not been documented.

The present study aimed at determining total and respiratory pathogen specific passive immune status at arrival and the timing of seroconversions against respiratory pathogens in white veal calves. Associations of the studied pathogens with BRD and carcass traits and the predictive value of the serostatus at arrival for subsequent BRD were explored. This information will be used to advise the veal industry on possible vaccination and risk classification strategies to be evaluated in the near future, for their potential to reduce BRD and the herewith associated antimicrobial use.

MATERIALS AND METHODS

STUDY DESIGN AND DATA COLLECTION

A cohort study was conducted in 15 commercial veal herds in Northern Belgium (2007-2009). Herds were conveniently selected based upon producers motivation to participate, and the sample was stratified on production type (5 dairy, 5 beef and 5 crossbred herds). Calves to sample were randomly selected using official stable lists upon arrival. The required sample size, based on the average size of the studied cohort of 390 calves, a 95% confidence to detect the presence of a pathogen and the average prevalence of respiratory pathogens set at 15% (based on the prevalence in diseased veal calves (Arcangioli et al., 2008; Pardon et al., 2011)), was 19 calves per herd (Winepiscope 2.0, University of Zaragoza, Spain). In 9 and 6 herds, 25 and 40 calves were sampled, respectively (467 calves in total). From all selected calves blood samples were taken from the jugular vein with a vacuum system (Venoject, Terumo) at 4 time points: arrival, 6, 12 and 24 weeks of production. Mortality (cause of death as determined by necropsy), morbidity and drug use were daily recorded on written treatment records by the producers. Criteria on which producers based individual treatment of an animal were as published previously (Pardon et al., 2012c). For BRD these criteria were mental state, appetite, nasal discharge, cough, rectal temperature (>39.5°C) and tachypnea. Hot carcass weight (HCW; kg) and other carcass traits were available from the slaughter houses. The methodology used to calculate carcass value (CV; in €¹) and carcass value diminished by treatment cost (CV-t; in €) is available elsewhere (Pardon et al., 2012c). Calves were group-housed on slatted floors after 6 weeks of individual housing. No calves were vaccinated against any pathogen.

SEROLOGICAL METHODS

Serum was collected within 8 hours after sampling and stored at -18°C until analysis. Semi-quantitative indirect ELISA's were used to detect antibodies (IgG1) against BRSV (whole virus), parainfluenza virus type 3 (PI-3) (whole virus), bovine viral diarrhoea virus (BVDV) (NS2-3 native protein), bovine adenovirus type 3 (BAV-3) (whole virus), BHV-1 (Respiratory ELISA kit pentakit, Bio-X Diagnostics) (whole virus) and *M. bovis* (*M. bovis* ELISA kit, Bio-X). Tests were performed and interpreted according to the

¹€1= approx.. US\$1.25, £0.80 at 27 May 2012

manufacturers prescriptions. A seroconversion was considered to have occurred if the signal increased by minimally two orders of magnitude (e.g. 0 to ++; + to +++ or ++ to ++++). Signals higher than + were classified as seropositive, whereas lower signals were regarded to be seronegative. Antibodies against *Mannheimia haemolytica* (whole cell) were determined at the laboratory of MSD Animal Health (Boxmeer, The Netherlands) with an in-house ELISA. Dilution series of the sera were incubated on plates and bound antibodies were detected after incubation with an anti-bovine serum-peroxidase conjugate. A four-fold titer increase was considered a seroconversion. BVDV antigen was detected by PCR in the arrival sera (Letellier and Kerkhofs, 2003). To confirm PI status a second PCR was performed at necropsy or on a serum sample of week 12 or 24. To determine total IgG (IgG1 and IgG2) concentration (g/L) in the serum samples as an estimate for passive immune status of the calves, a commercial competitive ELISA was used according to the manufacturer's instructions (ELISA kit for bovine immunoglobulin assays, Bio-X).

DATA MANAGEMENT AND STATISTICAL ANALYSIS

Mortality, treatment and carcass data were entered in a relational data base (Access 2007, Microsoft Inc.) and transferred to SAS version 9.3 (SAS Institute Inc., Cary, NC) for descriptive and statistical analysis. IgG levels below 10 g/L and 8 g/L were considered as insufficient and highly insufficient, respectively, both suggesting FPT (Van Donkersgoed et al., 1993; Hässig et al., 2007; Stilwell and Carvalho, 2011). Treatment records of 5 herds showed inconsistencies with calf identification, leaving 342 calves for analysis. A calf was considered morbid when individually treated at least one day for the respective disease. The unit of analysis was the individual calf. To evaluate associations between the predictor variables of interest (serostatus for respiratory pathogens and total IgG levels at arrival, seroconversion for respiratory pathogens in the first 6 weeks after arrival, breed, gender, age at arrival and diarrhea) and the BRD risk, logistic regression was performed, using a generalized linear mixed model (PROC GLIMMIX) with binomial distribution and logit link function with Wald's statistics for type 3 contrasts. As recommended by other researchers, predictor and outcome variables with a prevalence <5% were not used in the logistic models (Gillman et al., 2009; Brscic et al., 2011). To assess the associations of the same predictor variables next to days on feed (DOF), otitis and arthritis with the continuous outcome variables (HCW, CV and CV-t) a

linear mixed model was built (PROC MIXED). Model building strategies were as described previously and herd was added as a random factor to account for clustering of calves within a herd (Pardon et al., 2012c). Briefly, first a univariable analysis was performed and predictors with $P < 0.20$ were withheld for the multivariable model. Multivariable models were built stepwise backwards gradually excluding non-significant variables. Significance was set at $P < 0.05$ and $P < 0.10$ was considered a trend. All biologically relevant two-way interactions of significant fixed effects were tested.

RESULTS

Calves were on average 17.9 (standard deviation (SD)= 5.1; range (R)= 6-41) days old at arrival. In all herds prophylactic antimicrobials were provided in the milk for the first 7-10 days. A BRD outbreak occurred in every herd, on average 2.9 (SD= 0.6) weeks after arrival. To control BRD an average of 4.4 (SD= 1.9; R= 2-9) oral group antimicrobial treatments per herd were installed, of which 77.3% in the first 12 weeks after arrival. Further details on drug use are described elsewhere (Pardon et al., 2012a). In addition to the group treatments, 15.5% (53/342) of the calves were individually treated for BRD (of which 81.1% in the first 6 weeks after arrival), 6.7% (23/342) for diarrhea, 2.0% (7/342) for arthritis and 1.5% (5/342) for otitis. During production, 4.1% (19/467) died. The majority died due to digestive diseases (ruminal disorders, enterotoxaemia or enteritis), whereas only 5 (26% of the losses) died due to pneumonia. Calves were slaughtered on average 202 days (SD= 12; R= 178-238) after arrival. Average HCW, CV and CV-t were 179.5 kg (SD= 32.9; R= 94-227), €1150 (SD= 443; R= 389-2268) and €1078 (SD= 447; R= 203-2259), respectively.

There was serological evidence of infection with PI-3, BAV-3 and *M. bovis* in all studied herds. Circulation of BVDV, BRSV, BHV-1 and *M. haemolytica* could be demonstrated on 93.3% (13/15), 80.0% (12/15), 53.3% (8/15) and 86.7% (13/15) of the herds, respectively. Seroconversion rates along the production cycle are given in Figure 1. Seroconversion against *M. bovis* (73.2% of the calves on average), BVDV (57.8%) and BAV-3 (53.1%) was most prevalent. With exception of *M. haemolytica* and BAV-3 most seroconversions occurred in the first 6 weeks after arrival, consistent with the BRD peak. In these first 6 weeks, 51.4% (240/467) of the calves seroconverted against at least one virus (37.5%, 9.2%, 1.7% and 0.2% against 1, 2, 3 or 4 viruses, respectively), whereas this was 79.2% (370/467) over the complete production cycle. Multiple viral infections in the first 6 weeks most frequently involved BVDV (75% (39/52) of the multiple viral seroconversions) and BAV-3 (67.3% (35/52)).

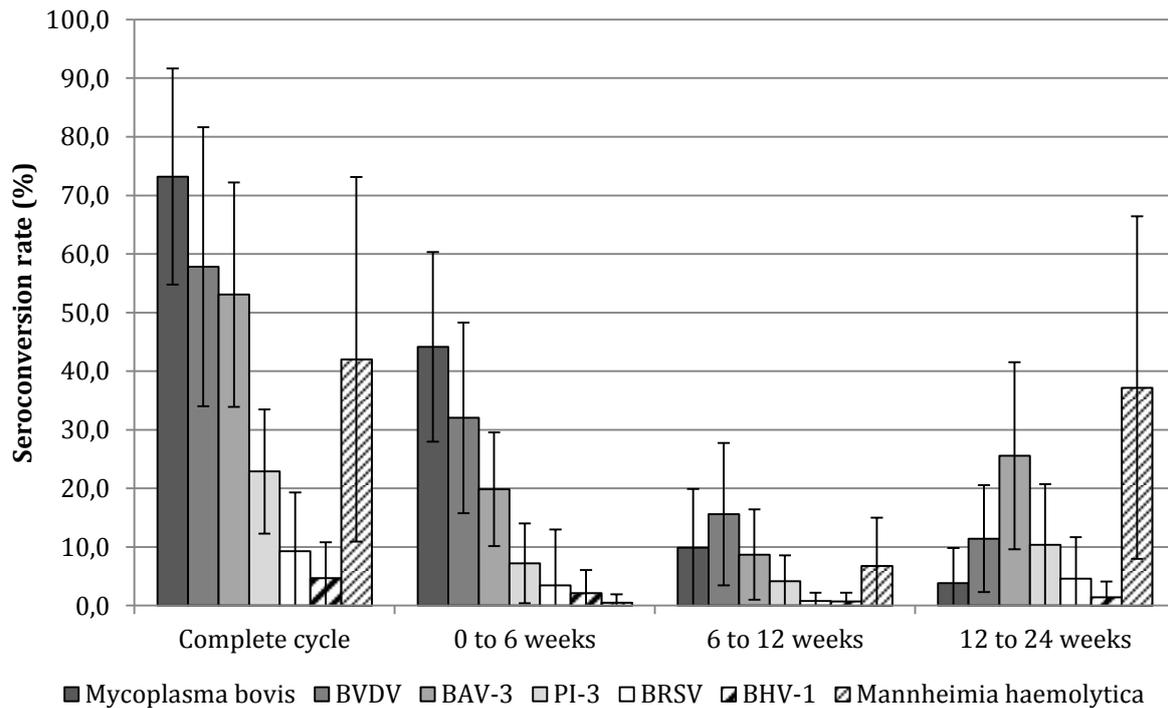


Figure 1. Seroconversion rates (%) for respiratory pathogens in different periods of the white veal production cycle. Bars represent standard deviation (467 calves, 15 herds, 2007-2009, Belgium).

The serological status for each pathogen at the different sampling points is given in Figure 2. At arrival, very few calves had antibodies against *M. bovis* (10.7% on average (R= 0-19.0%)). Along the production cycle a marked increase in the number of seropositives was noted for *M. bovis*, whereas this number decreased for BHV-1 and BRSV and remained status quo for BVDV, BAV-3 and *M. haemolytica* (Figure 2). The variation in seropositives at arrival between the herds was limited (coefficient of variation (CV) <20%) for all studied pathogens, with exception of *M. bovis* (CV=50%) and BHV-1 (CV=34%). For all studied pathogens, calves which were classified seropositive at arrival were less likely to seroconvert in the first 6 weeks after arrival (Table 1). Only for *M. haemolytica*, this effect lasted until 12 weeks after arrival. BVDV was the only virus for which seropositives at arrival had higher odds to seroconvert between 12 and 24 weeks after arrival (Table 1). Mean IgG concentration at arrival was 12.0 g/L (SD= 5.4; R= 1.8-31.2). IgG concentration and age at sampling were not significantly associated.

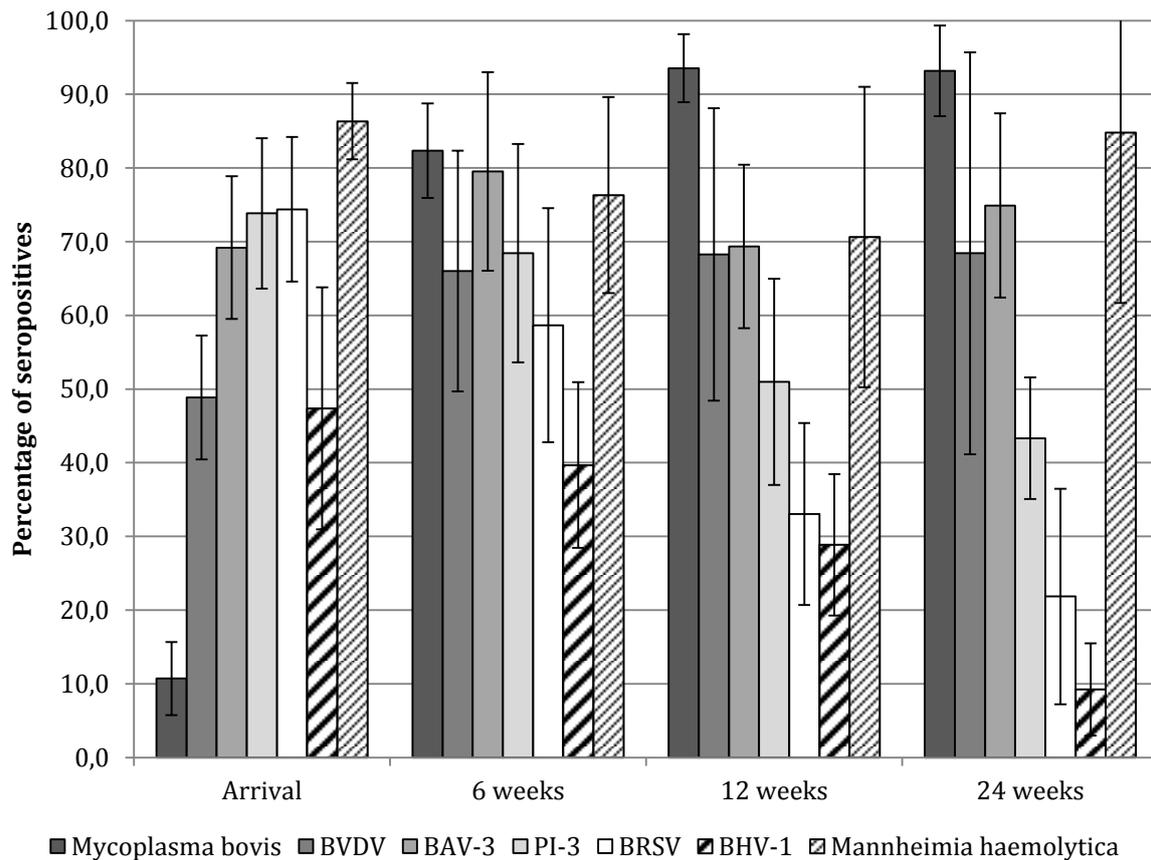


Figure 2. Seroprevalence (% seropositives) for respiratory pathogens at different stages of the white veal production cycle. Bars represent standard deviation (467 calves, 15 herds, 2007-2009, Belgium).

Based on a cut-off value of 10 g/L and 8 g/L, the mean prevalence of FPT was 40.1% (SD= 11.7; R= 24.0-59.9) and 26.8% (SD= 11.3; R= 12.0-44.0), respectively. The serostatus for respiratory pathogens at arrival was associated with the amount of total IgG, in this way that calves with IgG levels higher than 10 g/L showed higher odds of being classified as seropositive at arrival for each studied respiratory pathogen. BVDV antigen was detected in 2.1% (10/467) of the calves at arrival in 5 herds, with a prevalence of PI calves of 0.6% (3/467) (3 herds). PI animals had very high mortality risks (66.6% vs. 4.1%).

In the final multivariable logistic regression model for the risk for BRD in the first 6 weeks after arrival, only being seropositive to *M. bovis* (OR= 3.5; 95% Confidence Interval (CI)= 1.5-8.6; $P < 0.01$) and seronegative to PI-3 (OR= 2.3; CI= 1.1-5.0; $P < 0.01$) at arrival remained associated with an increased BRD risk. A trend towards a higher BRD

risk in calves with IgG levels <8 g/L was noticed (OR= 1.9; CI= 0.9-4.1; $P= 0.09$). This model was of very limited use for BRD prediction (sensitivity= 50.0% and specificity= 87.9%; cut off= 0.21; Figure 3). No associations between serostatus at arrival or seroconversion and HCW, CV and CV-t could be detected. HCW was higher in beef breeds ($P<0.01$) and in male calves ($P<0.01$), increased with DOF ($P<0.01$) and was negatively influenced by BRD ($P<0.01$) and diarrhea ($P<0.05$). The same effects were noticed for CV and CV-t with exception of the gender effect.

Table 1. Effect of antibody status at arrival on subsequent seroconversion rates to respiratory pathogens in white veal calves

Pathogen	Period ^a (weeks)	Seroconversion rate (%) (number of seroconversions/ number of calves)		OR ^b	95% CI	P
		Seronegative at arrival	Seropositive at arrival			
<i>Mycoplasma bovis</i>	0-6	52.7 (217/412)	1.9 (1/53)	0.01	< 0.01-0.09	< 0.01
	6-12	12.3 (50/406)	7.7 (4/53)	0.45	0.15-1.38	0.16
	12-24	5.3 (21/396)	2.1 (1/48)	0.31	0.04-2.44	0.26
	0-24	81.6 (336/412)	34.0 (18/53)	0.05	0.02-0.11	< 0.01
BVDV	0-6	57.2 (139/243)	2.7 (6/222)	0.01	<0.01-0.03	< 0.01
	6-12	14.6 (35/239)	16.0 (35/219)	1.01	0.59-1.72	0.97
	12-24	6.5 (15/232)	15.5 (33/213)	2.60	1.35-5.03	< 0.01
	0-24	75.7 (184/243)	34.2 (76/222)	0.06	0.03-0.11	< 0.01
BRSV	0-6	11.6 (13/112)	0 (0/353)	-	-	-
	6-12	1.8 (2/111)	0.6 (2/348)	0.32	0.04-2.27	0.25
	12-24	8.5 (9/106)	2.7 (9/338)	0.28	0.10-0.78	0.01
	0-24	22.3 (25/112)	3.7 (13/353)	0.15	0.07-0.31	< 0.01
PI-3	0-6	24.8 (29/117)	0.9 (3/348)	0.02	<0.01-0.08	< 0.01
	6-12	6.1 (7/115)	3.8 (13/344)	0.56	0.21-1.49	0.25
	12-24	9.8 (11/112)	9.3 (31/333)	0.89	0.41-1.92	0.77
	0-24	43.6 (51/117)	14.4 (50/348)	0.20	0.12-0.33	< 0.01
BAV-3	0-6	47.2 (68/144)	8.7 (28/321)	0.10	0.06-0.17	< 0.01
	6-12	11.8 (17/144)	6.6 (21/319)	0.51	0.26-1.03	0.06
	12-24	24.5 (34/139)	23.2 (71/306)	0.95	0.58-1.56	0.84
	0-24	77.8 (12/144)	39.9 (128/321)	0.16	0.10-0.26	< 0.01
BHV-1	0-6	3.4 (8/234)	0 (0/232)	-	-	-
	6-12	1.3 (3/232)	0 (0/226)	-	-	-
	12-24	1.8 (4/227)	0.5 (1/218)	0.28	0.03-2.62	0.26
	0-24	6.8 (16/234)	0.9 (2/232)	0.15	0.03-0.69	0.01
<i>Mannheimia haemolytica</i>	0-6	5.1 (3/59)	0 (0/402)	-	-	-
	6-12	14.0 (8/57)	5.0 (20/399)	0.32	0.12-0.81	0.01
	12-24	45.5 (25/55)	33.8 (132/390)	0.55	0.27-1.13	0.10
	0-24	57.6 (34/59)	36.6 (147/402)	0.26	0.12-0.56	< 0.01

^aSeroconversion between week 0 and 24 accounts for seroconversions between week 0 and 12 as well;
^bodds ratio for seroconversion in seropositives compared to seronegatives; -: not estimable; BVDV: bovine viral diarrhea virus; BRSV: bovine respiratory syncytial virus; PI-3: parainfluenzavirus type 3; BAV-3: bovine adenovirus 3; BHV-1: bovine herpesvirus 1; OR= odds ratio; CI= confidence interval

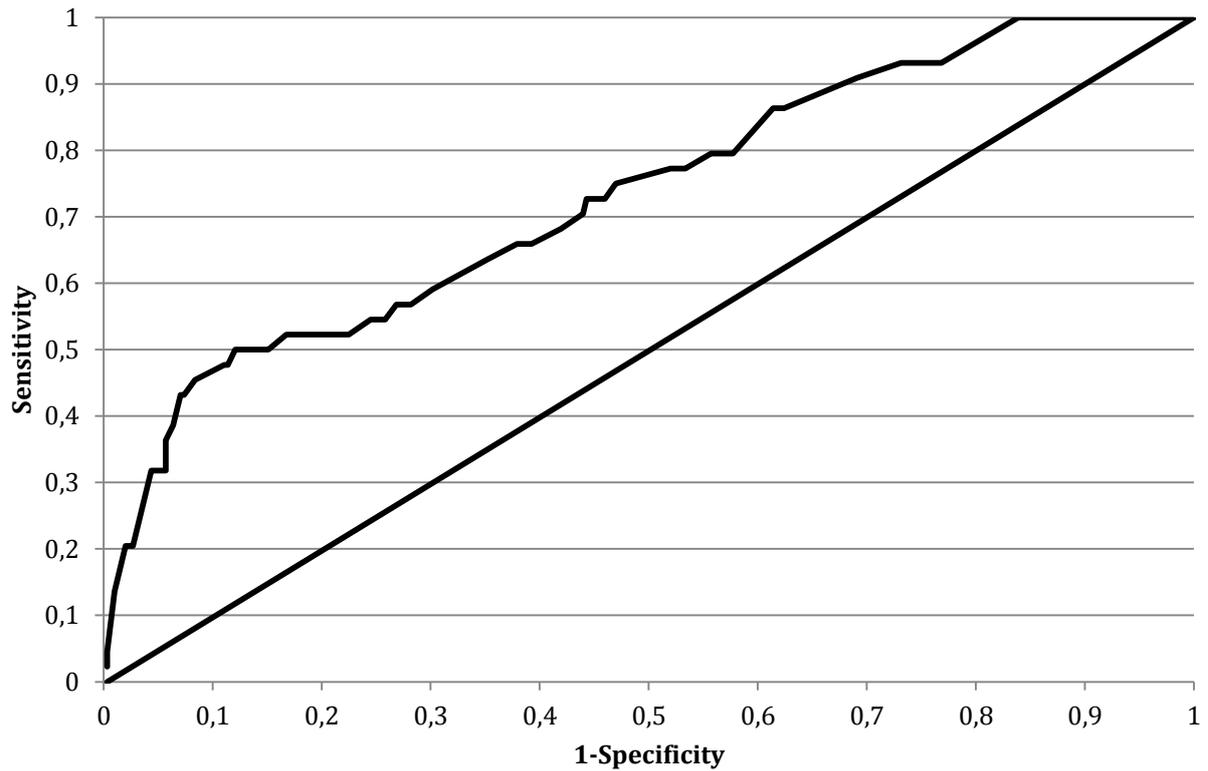


Figure 3. Receiver operating characteristic (ROC) curve for the prediction of BRD in the first 6 weeks after arrival, based on a model containing the serostatus for *Mycoplasma bovis* and PI-3 at arrival.

DISCUSSION

The coverage by oral antimicrobials in the first weeks after arrival, hampers the study of BRD and other diseases in the veal industry (Brscic et al., 2012). Calves that would have become ill remain undetected when applying metaphylaxis. Therefore, disease incidences in the present study are underestimated, and the identified associations should be interpreted as involving animals with obvious clinical symptoms or onset of disease before the group treatment.

Compared to systems which commingle older cattle, seroconversion rates to BRSV, PI-3 and *M. haemolytica* in the first weeks after arrival were much lower in veal calves, thanks to maternal immunity (Martin et al., 1999; Fulton et al., 2000; Assie et al., 2009). Maternal immunity does not prevent infection, but has been shown to reduce clinical symptoms and pathogen excretion under experimental conditions (Marshall and Frank, 1975; Makoschey et al., 2012). Presence of maternal immunity reduced the probability for seroconversion in the first 6 weeks after arrival. Due to suppression of humoral responses in calves with high maternal immunity, presence of the studied pathogens on the negative cohorts cannot totally be excluded. The gradual decline of maternal immunity, the prophylactic arrival medication and the gradual nasal colonization by *M. bovis* reaching a peak incidence 1 month after arrival are explanatory factors for the occurrence of the BRD peak at 3 weeks after arrival in veal calves, compared to in the first week after arrival in older cattle (Assie et al., 2009; Soehnlen et al., 2012).

Consistent with findings in diseased veal calves, *M. bovis* and BVDV were the dominant pathogens associated with the BRD peak incidence (Arcangioli et al., 2008; Pardon et al., 2011). Despite that a meaningful proportion of calves seroconverted to *M. haemolytica*, this occurred after 12 weeks and was not associated with BRD. Also in beef calves this spontaneous seroconversion was observed after the decline of maternal immunity, and this has been attributed to asymptomatic colonization of the nasopharynx (Prado et al., 2006). For most viruses, especially BVDV, infection continues at a lower level after the BRD peak. Next to the presence of older calves in certain cohorts, repeatedly reorganizing the calves according to drinking speed creates contact between infected and susceptible animals, when maternal immunity is waning. The prevalence of PI calves was higher than in Belgian youngstock (0.3%) or feedlot cattle at arrival (0.2%)

(O'Connor et al., 2005; Sarrazin et al., 2012). Possibly, this is due to the young age of veal calves and the deliberated selling of known positives to this industry. PI calves had low survival chances as documented in feedlots, but this should be interpreted carefully given the limited number of PI's in the present study (Loneragan et al., 2005). Excluding PI animals from veal production by testing them at arrival holds the risk of euthanizing transiently infected animals and will not prevent spread of the virus to susceptible calves. Identifying PI animals by ear notch at the herds of origin before purchase is likely a better strategy. Transient BVDV infection could not be associated with individual BRD treatment or carcass weight loss, in contrast to feedlot cattle in the arrival period (Martin et al., 1999). In fact no pathogen remained directly associated with HCW or CV, when accounting for BRD treatment. This suggests that not solely contact with a specific pathogen, but rather the infection pressure confronting the calf and its level of passive and acquired immunity determine whether or not pneumonia develops. Afterwards, the severity of the pneumonia and the efficacy of the installed treatment directly determine carcass losses.

A worrisome reality is that on average 40.1% (24.0-59.5%) of Belgian veal calves has insufficient (maternal) immunoglobulin levels at arrival, which is no different from the situation in the North American veal industry (Wilson et al., 2000). Apparently, dairy or beef producers do not deliver colostrum to calves destined for veal production with the same care as they do for their own livestock (Beam et al., 2009; Waldner and Rosengren, 2009). The clear association between FPT and an increased BRD risk as seen in conventional calves, could not be demonstrated in studies on veal calves (Postema and Mol, 1984; Postema et al., 1987). The older age of the calves at arrival or inaccurate calf birth dates provided by the herds of origin might have played a role. More likely the infection pressure is so high in veal calves, that it even causes disease in calves with adequate levels of maternal immunity. It might also be the other way around, namely that the continuous antimicrobial coverage is highly efficacious in protecting calves with FPT from disease. If the latter is true, animal welfare might be endangered when not using antimicrobials in groups of calves with such a high prevalence of FPT (Berge et al., 2005).

The percentage of seropositives to PI-3 and *M. bovis* at arrival was markedly larger (73.8% vs. 32.7% and 10.7% vs. 2.2%, respectively), compared to French veal calves,

whereas it was similar for BRSV (Arcangioli et al., 2008). Also, compared to a 1985 study on a limited number of veal herds in Belgium, the prevalence of maternal antibodies to BRSV and PI-3 has markedly increased, whereas it remained status quo for BVDV (Wellemans et al., 1985). These differences stress the importance of local and temporal data for the veal industry for decision making on vaccination. Generally, the predictive value of respiratory antibody levels at arrival for subsequent BRD was very limited, in contrast to feedlot cattle (Martin et al., 1999). Calves, seronegative to PI-3 at arrival, had increased BRD risks, again in contrast to feedlot cattle (Martin et al., 1999). It might signify the importance of PI-3 in causing more obvious clinical signs in neonatal calves or might point on the fact that calves seroconverting to PI-3 develop BRD earlier, before installment of the metaphylactic group treatment. The increased BRD risk in *M. bovis* seropositives at arrival was surprising. Since *M. bovis* antibodies can already be detected 6-10 days after infection, these calves might already have developed *M. bovis* pneumonia at the herd of origin resulting in higher odds to be treated in the veal herd (Uhaa et al., 1990). Under the present antimicrobial management, the obtained BRD risk models were of no practical use (low sensitivity) and the respiratory status at arrival was highly similar in each herd. Therefore, systematic determination of the serostatus to respiratory pathogens at arrival cannot be advised. However, determining total IgG and *M. bovis* serostatus at arrival, can help to identify herds of origin, with a bad colostrum management or infected with *M. bovis*. These factors can be used to avoid commingling of low risk calves with high risk calves in the veal supply chain, to adjust arrival management and to set calf purchase prices.

Despite high levels of maternal immunity, vaccination is highly advisable, given viral seroconversion in over 50% of the calves. Studies determining the efficacy of different vaccination strategies in reducing the BRD risk in veal herds urgently need to be performed. The most ideal situation would be that veal calves already have high levels of maternal antibodies to all respiratory pathogens at arrival (homogenisation). This can only be obtained by assuring the adequate delivery of colostrum from dams, vaccinated to all respiratory pathogens, at the herds of origin.

CONCLUSION

M. bovis and BVDV are the dominant pathogens in the BRD complex of veal calves. Despite high levels of maternal antibodies to all pathogens, except *M. bovis*, more than 50% of calves seroconvert to a virus in the BRD peak period. Excluding PI animals, assuring adequate colostrum uptake and vaccination are likely beneficial, but need confirmation in field studies. The predictive value of the serostatus at arrival for subsequent BRD risk or carcass traits was of no practical use. To help improve the validity of epidemiological studies in the veal industry, standard case definitions should be provided and group antimicrobial treatments avoided.

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CHAPTER 6

GENERAL DISCUSSION

INTRODUCTION

Because of the lack of epidemiological knowledge on veal calf diseases, the subsequent empiric antimicrobial use and the emergence of antimicrobial resistance, the overall objective of this doctoral thesis was to gain insights into current practices, into the epidemiology of morbidity and mortality in the Belgian white veal industry and into the underlying pathogens of respiratory disease. In this general discussion, a first paragraph is dedicated to the challenge that reduction of antimicrobial use poses to the veal industry. Next, the value of the applied study methodology for veal calf health monitoring systems and future holistic studies is discussed. With the obtained insights into the epidemiology of veal calf diseases (predominantly bovine respiratory disease (BRD)) in mind, the general discussion mainly aimed at critically reflecting on the current (medical) management practices. A series of possible short and long term measures to control BRD and reduce antimicrobial use, to be evaluate in the future, is proposed.

THE CHALLENGE OF REDUCING ANTIMICROBIAL USE

Before onset of the present doctoral thesis, it was suspected that antimicrobial use in the Belgian veal industry would be high, given the physiological possibility of easily applying oral medication to non-ruminating calves and the many stressors in the first weeks of production. That it would be as high as observed was beyond expectance. Based on their actual live weights at treatment, on average 416.8 veal calves out of 1000 were treated daily with a standard animal dose during the production cycle (Pardon et al., 2012a). This is 154.4 daily doses per animal year or 42% of their lives at the veal farm, corrected to approximately 25% of their lives, when accounting for multiple drugs given on the same day. The variation in drug use was considerably large ($SD= 148.4$ ADD/1000 calves, ranging from 123.4 to 818.9), illustrating that veal can successfully be produced with less antimicrobials. However the factors driving this low antimicrobial use remain to be determined and it is important to take production and welfare results of those farms into account.

It is clear that the veal industry faces an enormous challenge in the reduction of antimicrobial use. Contemporary medical management can no longer be justified with

current knowledge on the spread and potential dangers of antimicrobial resistance in humans and animals (Hammerum and Heuer, 2009; van Cleef et al., 2011). The typical organization of the veal industry, which inherently implies high degrees of commingling, likely makes the challenge much more greater in veal calves, compared to pigs and poultry, which maintain more closed herds. It is indeed important to notice that the veal industry works with very young calves, which are often immunologically deficient (40.2% of calves had insufficient IgG levels at arrival in Belgium) and are held within an artificial anaemic state, making them highly susceptible for disease (Stull and McDonough, 1994; Wilson et al., 1994, 2000; Pardon et al., 2012d). This fact, together with the multiple origin of calves, recent transport (including commingling at a sorting centre) and the obligatory group housing, factors which are all known to increase the disease risk substantially, make disease unable to overcome and the use of antimicrobial treatments necessary to guarantee adequate production results and animal welfare (Maatje et al., 1993; Gummow and Mapham, 2000; Pinchak et al., 2004; Sanderson et al., 2008; Step et al., 2008).

The relatively low mortality risk in comparison with for example the North American large scale dairy industry and current production results show that producers are reasonably able to care for these immunologically deficient calves under current management practices (Sivula et al., 1996; Pardon et al., 2012b; Walker et al., 2012). Nevertheless, severe production losses are still detected in calves, which required individual treatment next to the group treatments (Pardon et al., 2012c). Also, the proportion of chronic BRD cases with or without arthritis is large, causing welfare issues and more pronounced economic losses (Pardon et al., 2012b,c). Another worrisome finding was that BRD outbreaks reoccurred shortly after an oral group treatment or even worse while under group treatment, suggesting therapeutic failure due to antimicrobial resistance or underdosing (Pardon et al., 2011). All these findings on top of the previously reported high levels of antimicrobial resistance in bacteria from veal calves, stress that the medical management of veal calves urgently needs to be modified towards more sustainable management strategies.

Reducing antimicrobial use to conserve therapeutic efficacy of antimicrobials, is a shared responsibility with other sectors and human medicine (one health principle). Food supply and food safety are no longer the primary consumer motivators. The

increased scale of animal production and the large distance between the life of the European consumer and the reality of farming practices, creates a constant societal concern on animal welfare and a demand for environmental friendly and sustainable production. The negative perception on intensive antimicrobial use can potentially seriously hamper veal consumption. Especially because social perception of white veal production remains compromised at the European level, despite numerous efforts towards group housing and improved welfare. To counter this evolution the veal industry will have to invest in research towards a more sustainable production of white veal, using less antimicrobials.

VEAL CALF HEALTH MONITORING AND HOLISTIC APPROACH

VEAL CALF HEALTH MONITORING

To obtain a representative sample from a population, study units should be randomly selected (Thrusfield, 1996; Dohoo et al., 2009). Because the main objective of the above described studies was to gain detailed insight into the treatment practices and causes of morbidity and mortality, a highly accurate and detailed data input was necessary. Therefore, and to avoid data loss, veal producers were conveniently selected, based upon motivation to collaborate, but independent from disease history of the herd. Despite these efforts, we still encountered data loss for morbidity estimation in one third of the monitored herds. Because the sample size included 5% of the population with more than 90% of the active veterinarians represented and because housing and feeding are highly standardized worldwide in this production system, the possible selection bias, caused by the convenience selection, is believed to be limited. If any selection bias is to be expected, it is likely that the herds in the study are the “better” herds since farmers’ motivation to collaborate and to put effort in this type of studies generally coincide with better management. Therefore, the present study results should be interpreted as indicative, but not representative for the complete Belgian veal industry at present, and it might be that in the total population the situation is even worse. On the other hand, by the current approach highly detailed information on current practices, mortality and morbidity was obtained, which can immediately be used in communication towards the sector on health management and the prudent use of antimicrobials.

Health monitoring systems have been advised in every food animal production system worldwide, but in reality, for cattle, they are only in use on a limited number of, mostly dairy, herds. Also, the data registration only involves easy collectable production parameters (e.g. milk yield, fertility, slaughter weights,...). Health parameters, and especially calf health parameters, are seldom collected, because of the extra labour associated with registration and due to the fact that economic consequences of calf health are, with exception of mortality, only noticed at long term. In Norway, calf health parameters are collected at the national level as part of the Norwegian Dairy Herd Recording System (Svensson et al., 2003; Gulliksen et al., 2009b,c). Also, when calf health records from this database were validated by clinical examination on farm, by using dehorning records or by comparing disease incidences from herds which confirm complete calf health records with those not confirming, large discrepancies (e.g. underestimation of 40% for neonatal diarrhoea) between the true incidence and the recorded incidence in the database were detected (Gulliksen et al., 2009c).

Standardization of health recording is difficult, but crucial for the interpretation of the obtained disease incidences. In this doctoral thesis we used producer based diagnosis to estimate the BRD incidence. Producer based diagnosis has been shown to underestimate the disease frequency compared to veterinary diagnosis or lesion inspection at slaughter (White and Renter, 2009). Nevertheless, most large-scaled epidemiological studies use producer based diagnosis, due to financial and practical limitations (Sanderson et al., 2008; Babcock et al., 2009; Schneider et al., 2009). In the present thesis, the registration of individual drug use was used to estimate disease incidence. Next to the underestimation by using producer based diagnosis, the frequent metaphylactic application of antimicrobials interfered with recognition of individual disease. It is important to notice that the disease occurrence as estimated in the above described studies are all measured under a blanket of prophylactic or metaphylactic antimicrobial therapy. Therefore, they most likely only represent the tip of the iceberg. It is essential for future studies aiming at identifying risk factors for disease that disease can be estimated as precise as possible. Therefore, it is highly advisable to replace oral group therapies by individual administrations after diagnosis.

To remain maintained in practice, a monitoring system should be simple, imply limited extra labour and should create interesting feed-back for the producer. Therefore, it is highly advisable to attach additional health monitoring to previously installed or legally

required recordings. We linked disease to drug use, since individual registration of drug use from arrival on will likely become obligatory in the future. Individual drug use depends on the personal nature (detection ability, motivation,...) of the producer (van der Fels-Klerx et al., 2011). Therefore, the sector-wide use of standardized definitions for disease is an advisable step to ensure more accurate data input. For diarrhea, otitis and arthritis, which all have obvious clinical signs, a case definition can be very simple. For BRD this is much more difficult, since many cases remain undetected by the producer (Timsit et al., 2010, 2011). By means of a scoring card, as provided in Figure 1, the producer can be assisted in disease detection. For BRD, measuring rectal temperature is an essential parameter, which is hardly ever done at present. By taking rectal temperatures a distinction between undifferentiated fever and BRD can be made, as routinely applied in the North American feedlots (Booker et al., 1999; Galyean et al., 1999).

SCORE	0	1	2	3
Rectal temperature (°C)	< 39,0		39.1-39.5	> 39.5
Cough	none	Induced, single cough	Induced repeated cough or occasional spontaneous cough	Repeated spontaneous cough
Nasal discharge	Normal, serous 	Excessive serous or unilateral trace of pus 	Bilateral trace of pus or excessive mucous 	Bilateral, excessive, mucopurulent 
Mental state	Normal 	Moderate depression, reacts after stimulation 	Separated, mostly recumbent, depressed 	Severe depression, recumbency 

Figure 1. Example of a clinical score card to assist veal producers in the detection of cases of respiratory disease (BRD). Each parameter is scored separately and the scores are summed for each calf. Calves with a total score of 4 should be observed closely, whereas animals with a score 5 or more are considered BRD cases and treated.

Additionally, including case definitions for success or failure at short and long term is warranted. For BRD, we used the definitions reoccurrent case (same episode) and relapse case (new episode) as parameters for long term treatment failure, when the calf was retreated respectively between 5 and 14, and more than 14 days after the last day under antimicrobial coverage (Assie et al., 2004). An additional parameter of interest might be the clinical status at day 2-3, to evaluate short term treatment failure. Also for BRD, lung inspection at slaughter, which has a higher sensitivity and specificity than clinical detection, might be of interest (Thompson et al., 2006; Schneider et al., 2009; White and Renter, 2009). In pig production these slaughterhouse lesion scores have shown to be of high value to evaluate animal health at the herd level (Maes et al., 2001).

In previous work on risk factors associated with mortality, an important influence of the pen level next to the compartment and herd level has been noted (Bähler et al., 2010). Due to the very frequent relocation of calves in different pens according to drinking speed, we could not include the pen level. Adding the pen level could add additional information on the spread of diseases, which is potentially important for the application of targeted metaphylaxis (see next paragraphs). Because of the extra labour associated with pen recording, we preferred not to include this level to keep the recording system as simple as possible, ensuring correct morbidity input. Also in feedlots, collection of individual data turned out to be more valuable than pen level morbidity counts (Booker, 2010). The recording of drug use on written records was experienced as laborious by the producers, potentially leading to unreliable recording, as demonstrated before (DeVincent and Reid-Smith, 2006; Gonzalez et al., 2010). The use of electronic systems (personal digital assistant) and integration of producer and veterinarian records are advisable to ensure better data input (DeVincent and Reid-Smith, 2006; Gonzalez et al., 2010). When replacing the written record by a digital system, the registration system as used in the present doctoral thesis, has potential for the instalment of sector-wide veal calf health monitoring. We have shown that the integration as a whole (more in specific the size) has a significant influence on drug use (Pardon et al., 2012a). To the same extent the integrations' influence has the potential of installing a health monitoring system in over 90% of the sector at relatively short notice.

HOLISTIC APPROACH

This sector-wide instalment of a veal health monitoring system will be a crucial tool in the challenge of reducing antimicrobial use. Not only can it be used to benchmark drug use, mortality and morbidity (identifying individual problem herds), it also provides reliable data to evaluate the value of several measures for the reduction of antimicrobial use. In this respect the terminology 'holistic approach' should be introduced. In the past, too many studies have either focused only on the short term effect of a certain measure, or/and did only look at a limited number of outcome parameters of interest (Catry et al., 2008; Rérat et al., 2011). With a holistic approach, evaluating the effects of a given measure on all relevant outcome parameters over the complete production cycle is meant. All parameters of interest can be monitored, but in essence monitoring should involve parameters representing calf health, economics, welfare, and drug use. At present, and given the limited knowledge on the emergence and spread of antimicrobial resistance in veal calves, it is advisable also to include antimicrobial resistance in indicator bacteria and Pasteurellaceae as a parameter. For calf health, the above described recording system and definitions in combination with systematic and standardized necropsies can be used. Economic parameters (hot carcass weight, meat colour, fatness degree, carcass value) are already collected at the slaughter house. To represent the economic efficacy of a given measure (net profit per animal), total costs can be subtracted from the carcass value. Weighing the animals at different time points and calculating the average daily gain, might offer additional information on economic effects, as practiced in feedlots (Gardner et al., 1999; Thompson et al., 2006). For animal welfare, a whole range of specific parameters on normal and abnormal behaviour has been proposed (Stull and McDonough, 1994; Bokkers and Koene, 2001). At first instance, these parameters are only likely to be collected under specific experimental circumstances, for example to evaluate housing systems (Bokkers and Koene, 2001). Hb levels, mortality, morbidity, relapse rates (chronic BRD and arthritis) and the presence of abomasal ulcerations at slaughter might be more accessible parameters to evaluate animal welfare at present (Ortiz-Pelaez et al., 2008).

Finally, for monitoring drug use, there is a need for standardized calculation methods. It is highly advisable to use the standard daily dose methodology, as was done in the present thesis (Grave et al., 1999; Jensen et al., 2004; Timmerman et al., 2006). However,

there are some pitfalls. As we have shown, both the used live body weight at treatment as the defined daily dose (ADD), greatly influence the obtained treatment incidences (TI) (Pardon et al., 2012a). To resolve the issue of the applied body weight, the use of a standard weight is one option, but by using the live weights at treatment more accurate TI's are obtained. Calculating TI's using standard weight curves, is achievable in large scaled, highly standardized production systems such as veal calves, as we demonstrated (Pardon et al., 2012a). At the European level, standard animal daily dosages should be provided for TI calculation. If not standardized, transparency in the calculation methods is essential.

A holistic approach is absolutely necessary to identify the management practices that will lead to the most sustainable veal production in Europe, implying less antimicrobial use, and assuring adequate production results and animal welfare. Instalment of a sector-wide health monitoring program in veal calves provides an ideal situation for the evaluation of different preventive and therapeutic protocols. In the next paragraphs an overview is provided of possible therapeutic and preventive measures to be evaluated in the future, based upon the insights obtained from this thesis and reflecting on the current management.

BOVINE RESPIRATORY DISEASE MANAGEMENT

There is a strict order in the three control measures for BRD, which are structural, preventive and therapeutic management. First, the structure of an industry, for example the veal calf supply chain, should guarantee delivery of optimally healthy calves at the beginning of a production cycle. When a number of stressors are inherent to the production system (e.g. commingling of calves), then additional preventive measures (e.g. vaccination, reducing the degree of commingling,...) will be necessary. Only, when structural and preventive management are unable to overcome disease, treatment should be installed, only when an outbreak occurs. This is the order to control BRD, not vice versa. One should not easily accept that disease cannot be overcome by preventive measures alone, subsequently relying on antimicrobial use. Because changes in therapeutic management are urgently warranted and more easy to obtain at short notice compared to structural and preventive changes in veal production, the therapeutic management is discussed first. Next an overview of possible preventive measures is provided, ordered chronologically, based on their likeliness and easiness to be evaluated and installed in the nearby future.

THERAPEUTIC MANAGEMENT

Reducing antimicrobial resistance in food animals is a highly complicated issue, and a lot more research is necessary to be able to fully control the problem in the future. Based on the available literature, the only irrefutable observation is that using antimicrobials leads to the selection of antimicrobial resistant bacteria (Tenover and McGowan, 1996; Berge et al., 2006; Jensen et al., 2006; David and Gill, 2008). Whether oral medication, underdosing or large spectrum antimicrobials lead to more resistance selection, is heavily discussed, but unclear at present. However, from a clinical perspective, injection therapy is likely advisable over oral medication, since effective antimicrobial doses in the animal are more consistently reached with injection therapy. When orally medicating calves, factors, such as difficulties with suspending antimicrobials in the milk and the reduced feed intake in diseased calves, can cause suboptimal antimicrobial levels in the patient (Buhman et al., 2000). Also, after oral medication, antimicrobial residues remain in the milk pipe lines causing environmental selection pressure. To what extent this contributes to the high levels of antimicrobial resistance in veal calves and their caretakers is currently unclear. As in other food animal species, in veal calves,

producers and veterinarians frequently want to administer two or more antimicrobials at the same time in their belief of an enhanced efficacy. The combination of antimicrobials of which the advantage of a combined use has not been evidenced to be superior to the single antimicrobial, should be discouraged, because of possible reduced efficacy due to competition between drugs for binding sites or by the interference of bacteriostatic drugs with bactericidal by inhibiting bacterial growth. Combining drugs leads to higher drug use levels in the same time frame and increased costs. With these reflections in mind, this paragraph is built on the principles of only applying antimicrobials when no other (management) alternatives are available (prudent use), using as few different compounds as possible and basing the decision to use antimicrobials on diagnostics.

In veal calves, BRD management is priority since it is the major health problem, accounting for the largest proportion of individual and group level drug use and having the largest impact on production parameters (Pardon et al., 2011, 2012a,b,c). On the therapeutic side, individually based diagnosis and treatment for BRD is thought to be most beneficial in regard to the development of antimicrobial resistance at the group level (Berge et al., 2005). However, little success can be expected of this strategy under the present organization of the industry, since previous studies, evaluating metaphylactic group treatment versus individual treatment, consistently show that metaphylactic treatment leads to less morbidity (when applied at arrival), less relapse, less growth loss and is less laborious (Cusack, 2004; Berge et al., 2005; Catry et al., 2008; Rérat et al., 2011). One reason is that many producers tend to treat BRD cases rather late in the disease process, when pulmonary lesions are evident (Timsit et al., 2010, 2011). In contrast, metaphylaxis guarantees treatment in the early stage of the disease in a large proportion of calves. Additionally, a significant proportion of animals with pulmonary lesions at slaughter, remained undetected during production and therefore untreated by the producer, resulting in weight loss (Wittum et al., 1996; Thompson et al., 2006; Schneider et al., 2009). Detection rates can be improved by using clinical score cards, as previously described for dairy calves (McGuirk, 2008). A clinical score card adapted to veal production is shown in Figure 1. It is important that also these score cards are validated for their ability to timely detect animals requiring antimicrobial treatment. Also newer techniques such as infrared thermography (Schaefer et al., 2007, 2011), data from automatic milk feeders (Svensson and Jensen, 2007) or ruminal temperature

boluses (Timsit et al., 2010, 2011) have been shown to successfully assist in the detection of BRD cases. Despite the high costs of investment, these techniques might turn out valuable for the veal industry, when evaluated on economic parameters. Also, acute phase proteins have been used for the early selection of which calf to treat with antimicrobials (Humblet et al., 2004; Nikunen et al., 2007; Svensson et al., 2007; Holland et al., 2011), but at present no molecule has been found capable of distinguishing a viral from a bacterial infection in cattle, which hampers the use as a reductive tool for antimicrobial use.

Individual clinical diagnosis based treatment should be the final objective, but this requires well-trained producers, willing to invest extra labour in individually detecting and treating calves. Due to the enormous change in mentality this requires, and based on the poor performance of individual treatment compared to metaphylaxis in poorly trained producers, the evaluation of metaphylactic treatment for BRD at calf arrival, which is the major risk period for BRD, is of primordial importance. In feedlots, no difference between preshipping and arrival medication with tilmicosin or oxytetracycline on health and performance could be demonstrated (Duff et al., 2000). In this respect calf arrival seems the most appropriate and practical achievable timing of metaphylactic treatment in veal calves. At present, calves already receive prophylactic antimicrobials at arrival (predominantly amoxicillin, colistin or sulphonamides-trimethoprim), but these are administered for diarrhea prevention, and are not effective against *Mycoplasma bovis*, the most prevalent pathogen in veal calf BRD (Pardon et al., 2011, 2012a,d). Of the four common pathogens involved in neonatal diarrhea, only enterotoxigenic *Escherichia coli* (ETEC) is targeted by the present antimicrobial treatment. Clinical disease (diarrhea) by ETEC is limited to the first week of life in calves and therefore unlikely to occur at the age of veal calf arrival (14 days old). Despite this fact, ETEC was detected in 2.2% of calves at arrival and in 4.6% of fatal enteritis cases (de Visser et al., 1987; Pardon et al., 2012b). This might suggest a younger age on arrival than legally allowed, but on the other hand asymptomatic shedding of *E. coli* F5+ and F17+ can occur up till the age of 20 days and many weeks, respectively (Nollet et al., 1999). Anyway, this low prevalence of ETEC does not justify a prophylactic treatment directed towards ETEC at arrival. *M. bovis* quickly spreads after calf arrival, predisposing the respiratory tract to secondary infection with Pasteurellaceae (Houghton and Gourlay, 1983; Gourlay and Houghton, 1985; Lopez et al., 1986; Soehnlen et al., 2012).

Therefore, installing a metaphylactic treatment on calf arrival, with a single antimicrobial, effective against *M. bovis* (see Table 1), might be beneficial at short and long term as demonstrated in feedlots (Cusack, 2004; Booker et al., 2007). Rérat and others (2011) reported equal efficiency of a single long acting injectable antimicrobial with multiple oral antimicrobial treatments at arrival in veal calves. However, this study was not performed under field conditions and was limited to the clinical period. Most promising for the future, is the application of targeted metaphylaxis, only in groups of calves in direct contact with diseased calves, which has the advantage that not all calves need to be treated, hereby reducing the number of daily antimicrobial doses (Catry et al., 2008). Also within the concept of risk classification on arrival, targeted metaphylaxis is practiced (see under preventive measures).

Next to reduction and better targeting of antimicrobial group treatments, also the administration of individual treatments by the producers needs to be modified. At present, a very large number of different antimicrobial compounds is used per herd, often in combination and the advised therapy length is frequently not respected (Pardon, 2011; Pardon et al., 2012a). The instalment of treatment protocols for individual treatment by the producer, based on veterinary advice, will already be beneficial in reducing the number of different individually administered molecules per herd. Also here, the use of beta-lactam antibiotics or sulphonamide-trimethoprim for BRD treatment should be discarded as a first choice option, given the high prevalence of *M. bovis* (Pardon et al., 2011, 2012d). Such protocol can consist of a three step approach as presented in figure 2. In this way, chronically ill animals can be timely identified. These animals are likely chronically infected by *M. bovis* and/or bovine viral diarrhoea virus (BVDV), therefore unresponsive to antimicrobial treatment and a continuous source of infection for other calves (Shahriar et al., 2002; Gagea et al., 2006a, b; Pardon et al., 2011, 2012b). It is advisable to move these animals to a hospital pen as practiced in feedlots. With the knowledge that additional treatment will increase treatment costs and the carcass value decreases with increasing number of BRD treatments, it should be questioned whether further treatment of these chronically ill animals is still economically justified (Pardon et al., 2012c).

Which antimicrobials to use? A contemporary question in line with the ongoing instalment of formularies in different European countries. The decision of which

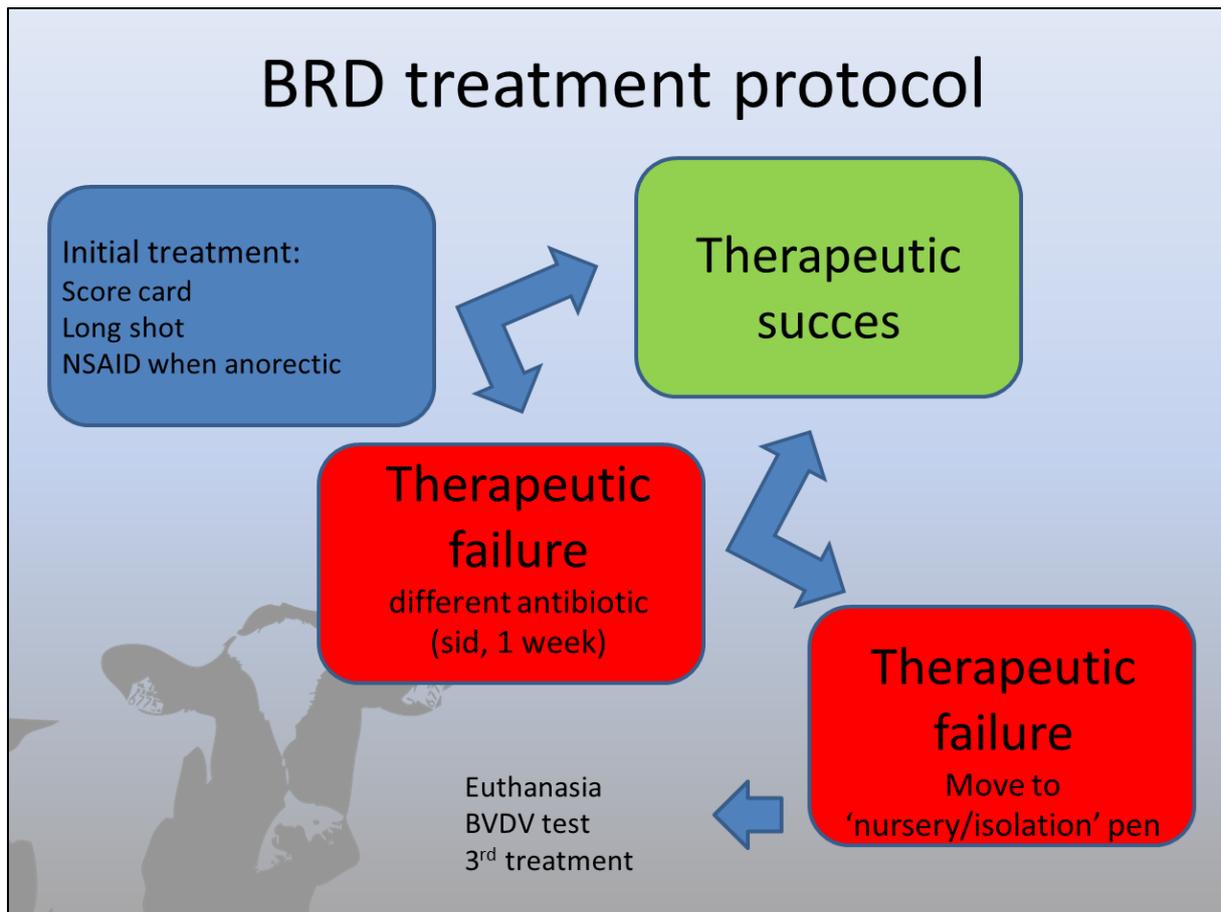


Figure 2. Schematic representation of a treatment protocol for individual treatment of bovine respiratory disease in white veal calves.

antimicrobials are preferably used as first, second or third choice should depend on the susceptibility of the target pathogen (including natural and acquired resistance), the clinical efficacy and the importance for human medicine (Table 1). However, price, withdrawal times and easiness of administration turned out to be greater motivators for product selection. In veal calves, antimicrobial therapy for BRD should be directed to *M. bovis* and Pasteurellaceae (Pardon et al., 2011, 2012d). *Mannheimia haemolytica* is present in the nasopharynx of diseased and healthy veal calves at the BRD peak and could be isolated from 10% to 20% of fatal pneumonia cases (Catry et al., 2005; Pardon et al., 2011, 2012b). On the other hand, the isolation rate of *M. haemolytica* from broncho-alveolar lavage samples during the BRD outbreak was only 4.4% in veal calves and the majority of seroconversions occurred after 12 weeks and were not associated with BRD outbreaks (Arcangioli et al., 2008; Pardon et al., 2012d). The exact

contribution of Pasteurellaceae to the veal calf BRDC requires further clarification, in order to support directing metaphylactic antimicrobial use towards these bacteria in veal calves.

Both for group as individual BRD treatment, a major issue for the selection of which antimicrobials to use, are the high levels of antimicrobial resistance in Pasteurellaceae in veal calves (Catry et al., 2005; Pardon et al., 2011). In the present doctoral thesis it has been shown that in particular herds the BRD incidence increases while calves receive oral antimicrobials, highlighting the potential of therapeutic failure due to multiresistant Pasteurellaceae or underdosing practices (Pardon et al., 2011). Previous work has shown that bacterial culture on nasopharyngeal swabs is fairly predictive of pulmonary infections in acutely ill calves, when used at the group level (Allen et al., 1991; DeRosa et al., 2000; Godinho et al., 2007). Therefore, culture on deep nasopharyngeal swabs seems a practical method to determine antimicrobial resistance in Pasteurellaceae to aid in the selection of effective antimicrobials for BRD treatment in respect to the classification of antimicrobials for human use (WHO, 2007). However, in beef herds with calves from multiple origin, different strains of *Pasteurella multocida* are present and their relative prevalence changes over time (Hotchkiss et al., 2010). Therefore, additional work is necessary to determine the required sample size, the animals to sample (diseased and/or healthy calves) and the best timing of sampling when using deep nasopharyngeal swabs to determine presence of bacterial pathogens and the different resistance profiles in a calf batch. Also, antimicrobial susceptibility testing for *M. bovis* might be of interest, but this is not standardized at present and should be interpreted with caution (Maunsell et al., 2011). Based on available studies, resistance to tetracycline, tilmicosin and spectinomycin is relatively frequent for *M. bovis* (Ayling et al., 2000; Thomas et al., 2003; Francoz et al., 2005; Rosenbusch et al., 2005). It has to be stressed, both for Mycoplasmata and Pasteurellaceae, that, in vitro antimicrobial resistance does not necessary mean clinical resistance, as particularly documented for macrolides (Godinho et al., 2005; McClary et al., 2011). A lot more knowledge on the epidemiology of antimicrobial resistance in veal calves is necessary. Given the all-in/all-out system in veal calves, it might seem achievable to reduce resistance levels at short term by using less antimicrobials. However, to what extent multiresistant Pasteurellaceae or Myco-

Table 1. Antimicrobial compounds, registered for treatment of respiratory disease in calves, and the natural and acquired resistances for respiratory bacteria isolated from veal calves in Belgium, classified according to their importance for human medicine

Antimicrobial compound ¹	Administration route	Importance for human medicine ²	Natural resistance for <i>Mycoplasma bovis</i>	Acquired resistance level (%) in <i>Mannheimia haemolytica</i>		Acquired resistance level (%) in <i>Pasteurella multocida</i>	
				Healthy calves ³	Diseased calves ⁴	Healthy calves ³	Diseased calves ⁴
Oxytetracycline ^a	PO, IV, IM, SC	II	No	100	73	70	33
Doxycycline ^a	PO	II	No	/	/	/	/
Florfenicol ^b	IM, SC	II	No	0	0	0	0
Trimethoprim-sulphonamides ^c	PO, IV, IM, SC	II	Yes	25	/	94	18
Lincomycin ^{d5} -spectinomycin ^{e5}	IM	III II	No		18		9
Procaine benzylpenicillin ^f	IM, SC	I	Yes	/	/	/	/
Amoxicillin ^f	PO, IM, SC	I	Yes	75	18	17	9
Amoxicillin-clavulanic acid ^g	PO, IM	I	Yes	/	/	/	/
Gentamicin ^h	IM	I	Yes ⁶	19	/	24	/
Tylosin ^d	PO, IV, IM, SC	I*	No	/	91	/	91
Tilmicosin ^d	PO, SC	I*	No	50	/	43	/
Tulathromycin ^{d5}	IM, SC	I*	No	/	/	/	/
Gamithromycin ^d	SC	I*	No	/	/	/	/
Tildipirosin ^d	SC	I*	No	/	/	/	/
Ceftiofur ⁱ	IM, SC	I*	Yes	0	/	0	/
Cefquinome ⁱ	IM, SC	I*	Yes	0	/	0	/
Flumequine ^j	PO	I*	No	/	18	/	24
Enrofloxacin ^k	PO, IV, SC	I*	No	13	18	0	9
Danofloxacin ^k	IV, IM, SC	I*	No	/	/	/	/
Difloxacin ^k	SC	I*	No	/	/	/	/
Marbofloxacin ^k	IV, IM, SC	I*	No	/	/	/	/

¹Antimicrobial compounds with identical letters show cross-resistance (>90%) in vitro. Dotted lines indicate partial cross-resistance. Differences in clinical efficacy might be present (Catry, 2005; Kadlec et al., 2011). Depending on the resistance gene, cross-resistance between the different macrolides and lincosamides might differ in this way, that the *erm(42)* gene induces cross resistance between erythromycin, tilmicosin, tildipirosin and clindamycin, but not tulathromycin or gamithromycin, whereas *msr(E)-mph(E)* conferred resistance to erythromycin, tilmicosin, tulathromycin and gamithromycin, but not to clindamycin and tildipirosin (Kadlec et al., 2011; Michael et al., 2012b). Please note that this table does not take multi-resistance due to a multi-resistance gene cluster acquired by plasmide transfer, as has also been described in Pasteurellaceae, into account (Katsuda et al., 2012; Michael et al., 2012a)

²Classification according to importance for human medicine: I*= prioritized critically important; I= critically important; II= highly important; III= important (WHO, 2009)

³Based on healthy veal calves in Belgium (Catry et al., 2005)

⁴Based on unpublished resistance data of 11 *M. haemolytica* and 11 *P. multocida* isolates from diseased veal calves in Belgium (Pardon et al., 2011)

⁵Registered for *Mycoplasma spp.*

⁶Efficacy documented to be very poor (Thomas et al., 2003)

PO= perorally; IV= intravenously; IM= intramuscularly; SC= subcutaneously

plasma strains, residing in the veal stables and surviving round after round, are present and able to infect newly arrived calves, remains to be determined. The latter might severely jeopardise reduction of antimicrobial resistance levels in the veal industry at short term.

Another issue is the lack of studies, evaluating the required duration of antimicrobial therapy to prevent relapse or active lesions at slaughter. This is absolutely necessary to optimize the treatment protocols and might be longer than expected given the high prevalence of *M. bovis*. In addition to antimicrobials the long term effects of the use of non-steroidal anti-inflammatory drugs (NSAID's) might be of interest. Several studies have shown the benefits of the use of NSAID's in the clinical period, but long term effects on production parameters have not been documented (Bednarek et al., 1999, 2003; Lockwood et al., 2003; Todd et al., 2007, 2010). Also other alternative (preventive) treatments, such as pre- and probiotics, merit evaluation for their ability to reduce antimicrobial drug use (Timmerman et al., 2005; Ripamonti et al., 2011).

PREVENTIVE MANAGEMENT

Regardless of the pathogens involved, prevention of pneumonia is the objective, since the latter and not only the contact with a given pathogen has been related to production loss (Schneider et al., 2009; Pardon et al., 2012c,d). Preventive management should be directed on reducing the infection pressure and stress. This reduction can be obtained at the fattening herd, but very likely an even more important effect can be expected by reducing stress and infection pressure in the veal calf supply chain, hereby assuring that calves arrive at the fattening herds in optimal conditions. An overview on possible preventive measures to be evaluated for their ability to reduce antimicrobial use, is given in the next paragraphs.

VACCINATION

A lot of hope has been set on vaccination to reduce disease and herewith associated antimicrobial drug use. Vaccination reduces pathogen transmission, the infection pressure and the associated pulmonary damage. A major obstacle for the effective use of vaccines is timely administration before periods of stress or exposure to pathogens. In feedlots the BRD morbidity risk is highest shortly after arrival and progressively

decreases near the end of the production cycle (Snowder et al., 2006; Babcock et al., 2009). Despite the fact that BRD cases already occurred at arrival, the BRD peak incidence in white veal calves was reached at week 3-4 (Pardon et al., 2012b). This time lag can be explained by the applied metaphylactic treatment, the gradual decline of maternal immunity or the incubation period of the different pathogens (Fulton et al., 2004). In feedlots, vaccination against viral agents was most effective when performed at the herd of origin, 45 days before shipment (Step et al., 2008). In veal calves, preshipment vaccination is hard to achieve given the large number of herds of origin per veal herd and the limited time calves spend on the herd of origin, but this would be the most appropriate timing of vaccination. Another major issue is that maternal immunity is still present at the age of arrival at the veal herd, potentially interfering with vaccination (Hässig et al., 2007; Pardon et al., 2012d). As a consequence, inactivated vaccines cannot reach a protective antibody response before the challenge, since it takes approximately 6 weeks for immunity to be established and vaccination responses are suppressed by maternal immunity (Makoschey et al., 2006). However, using inactivated vaccines by injection might have its benefits, even in calves with maternal immunity against bovine respiratory syncytial virus (BRSV), since a single injection with an inactivated BRSV vaccine primed cellular immunity and resulted in reduced nasal virus excretion and lesser pulmonary lesions (van der Sluijs et al., 2010). Intranasal vaccination with live vaccine, can breach maternal immunity, stimulating local immunity and seems a better option to protect neonatal calves (Ellis et al., 2010). Vaccination at arrival, involving parainfluenzavirus type-3 (PI-3) and BRSV intranasal or BVDV, bovine herpesvirus 1 (BHV-1), BRSV and PI-3 by injection is currently implemented in some French and North American veal cohorts, respectively (Arcangioli et al., 2008; Soehnlen et al., 2012). In Belgium and the Netherlands vaccination is not generally practiced for economic reasons and because of the empirically experienced lack of efficiency.

As documented in the present thesis, almost all respiratory pathogens are present in each veal cohort, due to the multiple origin of the calves (Pardon et al., 2011; 2012d). Since *M. bovis* is the dominant pathogen and few calves have maternal immunity against *M. bovis* at arrival, vaccination against this pathogen seems promising (Arcangioli et al., 2008; Pardon et al., 2012d). Unfortunately, there are no *M. bovis* vaccines registered in Europe. Despite an older report on a clinically efficient vaccine under experimental conditions, the available vaccines in the United States showed very low efficacy in recent

field studies (Nicholas et al., 2002; Maunsell et al., 2009; Soehnlen et al., 2011). To assure homogenous respiratory immunity in the calves, vaccinating against as many respiratory pathogens as possible is advisable. However, when there are economic restrictions, it is difficult to set priorities. Based on the observed maternal antibody levels at arrival, necropsy results, seroconversion rates and associated BRD risks, vaccination against BVDV, BRSV and PI-3 is likely of higher priority than BHV-1 and *M. haemolytica* (Pardon et al., 2011, 2012b,d). BVDV vaccination is only advisable, when exclusion of PI animals is not possible, and given the conflicting results from feedlot studies, it can be questioned whether BVDV vaccination at arrival will reduce the BRD risk in veal operations (Loneragan et al., 2005; O'Connor et al., 2005).

There are reasons to doubt on the benefits of vaccinating stressed calves, at the time they are confronted with a whole series of pathogens. Studies determining the efficacy of vaccination at the herd of origin or at arrival (intranasal or by injection) in veal herds are currently not available. They urgently need to be performed to provide scientific data on the achievability and efficacy of these preventive measures.

MATERNAL IMMUNITY

Another possibility to offer protection to the calves is by providing adequate maternal immunity. For most respiratory pathogens maternal antibodies do not prevent infection, but offer partial protection, resulting in less severe lesions (Marshall and Frank, 1975; Cowan and McBeath, 1982; Kimman et al., 1988; Belknap et al., 1991; Makoschey et al., 2012). We could only associate the presence of maternal antibodies against PI-3 with protection from individual treatment for BRD (Pardon et al., 2012d). An important remark is that the applied detection system for BRD, was based on producer based diagnosis in calves that are frequently treated by antimicrobials, and that very likely not all BRD cases have been detected. Therefore, existing relationships between maternal antibody levels against some respiratory pathogens and BRD risk might not have been identified in that study (Pardon et al., 2012d). Interestingly, providing the colostrum of dams vaccinated with an inactivated vaccine based on iron regulated protein of *M. haemolytica* offers partial protection to the calves after challenge (Makoschey et al., 2012). By vaccinating the dam and providing adequate colostrum to the calves, a homogenous maternal immunity in a calf batch can be achieved at arrival, likely reducing excretion and circulation of pathogens in veal calves.

A worrisome reality is that on average 40.1%, ranging from 24.0-59.5%, of Belgian veal calves has insufficient (maternal) immunoglobulin levels at arrival, which is no different from the situation in the North American and Canadian veal industry (McDonough et al., 1994; Wilson et al., 2000). Apparently, dairy or beef producers do not deliver colostrum to calves destined for veal production with the same care as they do for their own livestock (Beam et al., 2009; Waldner and Rosengren, 2009). Failure of passive transfer (FPT) has been associated with increased morbidity (diarrhea, BRD and septicaemia) and mortality risk in different production systems (Donovan et al., 1998; Lofstedt et al., 1999; Waldner and Rosengren, 2009; Stilwell and Carvalho, 2011). We could not demonstrate an association between total IgG levels and mortality, likely due to the intensive antimicrobial treatments and the limited number of mortalities in the first weeks after arrival in that prospective study (Pardon et al., 2012d). More surprising, there was also no significant relationship between IgG levels at arrival and individual treatment for diarrhea or BRD (only a trend for a higher BRD risk in calves with IgG <8g/L) (Pardon et al., 2012d). An underestimation of disease due to a suboptimal detection system, hampered by the frequent metaphylactic group treatments, is one possible explanation. The older age of the calves at arrival or inaccurate calf birth dates provided by the herds of origin might also have played a role. On the other hand, also in previous, less extensive, studies in veal calves no link between IgG levels and morbidity could be shown, which is in contrast with other production systems (Postema and Mol, 1984; Postema et al., 1987). Possibly the infection pressure is so high in veal calves that it even causes disease in calves with adequate levels of maternal immunity. It might also be the other way around, namely that the continuous antimicrobial coverage is highly efficacious in protecting calves with FPT from developing disease. If the latter is true, animal welfare might be endangered when not using antimicrobials in groups of calves with such a high prevalence of FPT (Berge et al., 2005). Comparing groups of veal calves with adequate immunoglobulin levels at arrival with groups with FPT might further clarify the role of FPT in veal calf diseases and drug use.

MYCOPLASMA BOVIS

Controlling or preventing *M. bovis* infection in veal calves appears to be essential, given the large proportion of calves seroconverting and the high incidence of chronic BRD and MbAD (Pardon et al., 2012b,c,d). Due to the absence of an efficient vaccine, other control

measures need to be implemented. In this respect, it is important to realize that the presence of *M. bovis* is not unable to be overcome as a risk factor for pneumonia, since disease expression is variable, and the bacteria can be isolated from healthy animals (Maunsell et al., 2011). The majority of veal calves will eventually become infected with *M. bovis*, most of them within the first weeks after arrival (Arcangioli et al., 2008; Pardon et al., 2012d; Soehnlen et al., 2012). To limit the consequences of this infection (both acute as chronic BRD and MbAD), controlling other pathogens and risk factors (environmental and calf immunity), predisposing for BRD, is essential. Whereas risk factors for acute BRD can be partly extrapolated from other production systems, there is currently little information on the risk factors for MbAD or for the development of chronic, unresponsive BRD. Reduction of the number of stressors and the degree of commingling in the veal supply chain (as documented below) is likely to positively contribute to the reduction of BRD cases in veal calves.

Alternatively, creating *M. bovis* negative cohorts by excluding positive calves at arrival might be a promising approach, but will be hard to achieve in practice. First of all, calves which are seropositive at arrival, and therefore possibly asymptomatic carriers of *M. bovis*, were present on almost each studied cohort, albeit at relatively low frequency compared to the other respiratory pathogens (Pardon et al., 2012d). Still this number (10.7% on average) is too high to economically justify exclusion of these calves from production by on arrival testing. Identifying positive herds of origin, and grouping calves from these herds in a limited number of veal herds, hereby preventing infection of a large number of veal cohorts might be a better option. However, before such attempts are made, more insights are necessary into the epidemiology of *M. bovis* in veal cohorts. It is not known whether *M. bovis* comes with every new calf batch, or resides in the environment of the veal stable, infecting newly arriving calves. The latter is very likely since after cleaning, disinfection and sanitary vacancy are hardly practiced in the Belgian veal industry and *M. bovis* can survive for quite a long time in organic material, despite the absence of a cell wall (Pfutzner and Sachse, 1996). Nevertheless, *M. bovis* is susceptible to most disinfectants and highly sensitive to higher environmental temperatures (Pfutzner and Sachse, 1996). Therefore, the benefits of disinfection and sanitary vacancy, to reduce the infection pressure and subsequently morbidity and drug use, as documented for BRD in French cow-calf farms, should be among the first measures to be evaluated in the holistic approach (Assie et al., 2004).

BOVINE VIRAL DIARRHEA VIRUS

Next to *M. bovis*, BVDV was the leading pathogen associated with BRD in veal calves (Pardon et al., 2011). Half of the calves had maternal immunity, whereas the other half didn't, resulting in a seroconversion rate of over 50,0% (Pardon et al., 2012d). Calves, which were seropositive on arrival, had lower odds to seroconvert in the first 6 weeks, but seroconverted anyway after maternal immunity had waned (Pardon et al., 2012d). Despite that no direct associations between transient BVDV infection and BRD could be demonstrated, it is clear that persistently infected (PI) animals are very likely to die in the veal industry from the consequences of chronic, unresponsive pneumonia (Pardon et al., 2012b,d). The same synergy between BVDV and *M. bovis* was noticed at necropsy in feedlots (Haines et al., 2001; Shahriar et al., 2002). Therefore, combatting BVDV might strongly reduce the consequences of *M. bovis* in veal calves. Two options are available: either vaccination of susceptible calves or exclusion of PI animals. Contact with a PI animal increased the BRD risk even in previously BVDV vaccinated cattle in a single feedlot (Loneragan et al., 2005). In contrast, more recent studies, also on BVDV vaccinated feedlot cattle, did not find a relationship between contact with a PI calf and BRD risk or reduced growth (O'Connor et al., 2005; Elam et al., 2008). In veal calves, vaccination before the risk period is highly difficult to achieve, certainly because only inactivated BVDV vaccines are available. Therefore, exclusion of PI animals seems more promising, and is also one of the priorities to be tested in practice. On arrival testing by antigen PCR is one possibility, but next to the very high costs, one risks of excluding transiently infected animals, as we demonstrated (Pardon et al., 2012d). Also, BVDV virus might already have spread in the calf batch before the PI animals can be excluded. Detection of PI animals at the herd of origin and subsequently excluding them from veal production, might be better achievable. With the recent implementation of BVDV antigen testing on ear notches at birth, the administrative possibilities are present to identify PI animals and BVDV positive herds.

RISK CLASSIFICATION

A promising approach to reduce antimicrobial use, while ensuring sustainable veal production, is disease risk classification at arrival. By classifying calf batches according to risk at arrival, metaphylactic treatment at arrival can be limited to calf batches at high risk, whereas treatment can remain at individual basis in low risk batches. This risk

assessment of calf batches is already in use in feedlots and different studies have been conducted to identify the relationship between so called entry characteristics and morbidity, mortality and performance (Moore et al., 2002; Cusack et al., 2007; Reinhardt et al., 2009; Booker, 2011). A low arrival weight, calf supplier, season of arrival and breed have all been associated with a higher BRD risk and mortality in feedlots (Moore et al., 2002; Cusack et al., 2007; Reinhardt et al., 2009). Recently, also in veal calves, it has been shown that lighter calves at arrival and calves arriving in autumn compared to winter have an increased BRD risk (Brscic et al., 2012). Whereas breed predispositions were identified for several diseases, we could not identify significant associations between the age at arrival and the studied parameters, as was the case in Swiss veal calves (Rérat, 2010). In feedlots, also preconditioning, which implies measures taken before shipment to the fattening herds (e.g. weaned for at least 30-45 days, vaccination, dehorning, castration, anthelmintic treatment, accustomed to feed bunks and water troughs), turned out to have great benefits for animal health and performance (Roeber et al., 2001; Fulton et al., 2002; Dhuyvetter et al., 2005). In different US states preconditioned calves are even certified (Duff and Galvayan, 2007). In veal calves there is little time for preconditioning since the calves are already transported at the age of 14 days old. Nevertheless, identification of calf suppliers (sorting center, tradesman and herd of origin), which deliver calves with increased morbidity and mortality risk can be highly valuable to the veal industry, to set purchase prices. An even better evolution would be that producers of the herds of origin receive higher purchase prices, when assuring adequate colostrum uptake, BVDV free calves or additional health measures such as intranasal vaccination or recordings on diseases in the first weeks of life (certification). Of course the benefits of these measures need to be confirmed in the holistic approach.

REORGANIZING THE VEAL CALF SUPPLY CHAIN

Finally, if the above mentioned measures cannot reduce antimicrobial use to an acceptable level, drastic changes in the veal calf supply chain are required. As extensively illustrated in the introduction of this thesis, the veal calf supply chain inherently holds an increased BRD risk due to the many stressors and the high degree of commingling. Especially the number of herds of origin per veal herd, with on average 1.3 calves per herd of origin, is unique in food animal production. Limiting the number of

herds of origin has been associated with reduced BRD risks in other production systems (Gummow and Mapham, 2000; Step et al., 2008). This is however practically not feasible in the veal industry. However, calf batches may be combined based on risk classification, to avoid commingling of low risk batches with high risk batches. Direct transport from the farms of origin to the veal herd is likely better than passage through a sorting center as documented in feedlots (Gummow and Mapham, 2000; Step et al., 2008). Also, limiting the length of the arrival period is of importance, with preference to filling the cohort within a week (Martin et al., 1982; Alexander et al., 1989). All-in/all-out systems, with no large age differences between different cohorts in a herd, are advisable (Gulliksen et al., 2009a). Transport conditions, especially transport duration, are major risk factors for BRD in different cattle production systems (Staples and Haugse, 1974; Cave et al., 2005; Sanderson et al., 2008). Many efforts, e.g. conditioned trucks, have been made by the veal industry, but their health effects have been hardly validated in studies. Also, the frequent relocation of calves in pens according to drinking speed, is potentially a crucial factor in disease spread. With the installment of a health monitoring system, the exact contribution of each of these factors to the BRDC of veal calves can be further elucidated.

CONCLUSION

The present doctoral thesis illustrated that reducing antimicrobial use forms a gigantic challenge to the veal industry, to be addressed at relatively short notice. BRD played a crucial role in morbidity, mortality and in the subsequent drug use. Therefore, to reduce antimicrobial use, focus should be on BRD control. Sector-wide instalment of a health monitoring program is the essential first step, in which veterinarians can play a central role. Protocollisation and training of the producers in terms of data collection, disease detection and drug administration are absolute necessities. The present documentation on antimicrobial use, health, mortality and production parameters can be used as a historical benchmark, from where to work towards better, more sustainable, management strategies. Based on the provided insights into the epidemiology and pathogenesis of BRD, reflections could be made on the current management and a whole series of therapeutic and preventive measures could be proposed. In the future, using a holistic approach, the management conditions favoring the most sustainable veal

production can be selected for each situation, hereby answering on the societal demand of animal welfare and environmental friendly food production next to food safety.

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SUMMARY

The veal industry is specialized in rearing male calves on a low iron milk diet, hereby obtaining white meat. As in other food animals, high levels of antimicrobial resistance have recently been detected in bacteria from veal calves, potentially threatening treatment options in both human as veterinary medicine. This worrisome observation is currently overwhelming veterinary medicine and revisions of current treatment practices (benchmarking) and the prudent use of antimicrobials in food animals are highly recommended by the European authorities.

As a general introduction (**Chapter 1.1.**), an overview of the typical characteristics and challenges of the Belgian white veal industry is given. A synthesis of the current knowledge on antimicrobial resistance in pathogenic, zoonotic and commensal bacteria from veal calves is provided. Bovine respiratory disease (BRD) is the leading cause of morbidity and mortality in young animals in almost every cattle production system worldwide. BRD is a multifactorial disease resulting from the complex interaction of pathogens with host factors (immunity) and the environment, and is therefore also termed bovine respiratory disease complex (BRDC). The BRDC differs in between production systems, and knowledge on the epidemiology and pathogenesis of BRD in each production system is necessary, in order to determine effective control measures for that particular production system. In **chapter 1.2.** an overview of the constitution of the BRDC in veal calves is given. From this literature review it could be concluded that, under the current organization of the industry, veal calves are at ultra-high risk of developing BRD, making antimicrobial use necessary to overcome disease. Hardly any information on the pathogens involved in the BRDC of veal calves is available.

For economic reasons and due to a lack of epidemiological knowledge on diseases in veal calves, veterinarians have been tied to empiric, often blind, treatment in the veal industry for decades. In line with this problematic situation and with the emergence of antimicrobial resistance, the overall objective of this doctoral thesis was to gain insights into current practices, into the epidemiology of morbidity and mortality in the Belgian white veal industry and into the underlying pathogens of respiratory disease (**Chapter 2**). This to provide the industry with objective data from where to install and evaluate sustainable preventive and therapeutic protocols, using less antimicrobials, in the future.

At present, no information on drug use in white veal calves in Belgium is available. Therefore, the first objective was to document antimicrobial and anti-inflammatory drug use in the Belgian veal industry (**Chapter 3**). A prospective study was installed, monitoring group and individual drug use on 15 veal cohorts in Belgium. Treatment incidences (TI) based on animal defined daily dose (ADD), prescribed daily dose (PDD) and used daily dose (UDD) were calculated. Antimicrobial drug use was highly intensive, with on average 416.8 ADD per 1000 animals at risk. Antimicrobial drug use consisted for 95.8% of oral group treatments, and only for 4.2% of individual administrations. BRD was the main indication for both group (53.0%) and individual drug use. The most frequently used antimicrobials (group treatments) were oxytetracycline (23.7%), amoxicillin (18.5%), tylosin (17.2%) and colistin (15.2%). Deviations from the leaflet recommendations were frequently encountered, with 47.3% of the group treatments underdosed (predominantly oxytetracycline and tylosin to treat dysbacteriosis). Based on the UDD, smaller integrations (less than 50 herds) used more antimicrobials than larger ones. Producers used higher dosages in group treatments than prescribed by the veterinarian in cohorts with a single caretaker. A large variety of antimicrobial compounds were used for individual treatments, including the critically important cephalosporins (8.0%) and fluoroquinolones (12.6%). Individual treatments were generally overdosed. Anti-inflammatory drugs were far less frequently used ($TI_{UDD} = 5.94$), mostly sodium salicylic acid in group treatments. Individually administered anti-inflammatory drugs were generally overdosed. The main conclusion was that antimicrobial drug use is highly intensive at present and that future reduction can only be achieved by reducing the number of oral group treatments.

In a second study (**Chapter 4.1.**), the objective was to determine the causes and epidemiology of morbidity and mortality in dairy, beef and crossbred white veal production in Belgium. A total of 5853 calves, housed in 15 production cohorts (5 per breed), were followed during one production cycle. Causes of mortality were determined by necropsy. Morbidity was daily recorded by the producers. The total mortality risk was 5,3% and was significantly higher in beef veal production compared to dairy or crossbreds. The main causes of mortality were pneumonia (1.3% of the calves at risk), ruminal disorders (0.7%), idiopathic peritonitis (0.5%), enterotoxaemia (0.5%) and enteritis (0.4%). Idiopathic peritonitis was identified as a disease of unknown origin, specifically occurring around 8-10 weeks after arrival. Belgian Blue

beef calves were more likely to die from pneumonia, enterotoxaemia and arthritis. Detection of bovine viral diarrhoea virus (BVDV) at necropsy was associated with chronic pneumonia and pleuritis. Of the calves, 25.4% was treated individually and the morbidity rate was 1.7 cases per 1000 calf days at risk. The incidence rate of respiratory disease, diarrhoea, arthritis and otitis was 0.95, 0.30, 0.11 and 0.07 cases per 1000 calf days at risk, respectively. Morbidity peaked in the first three weeks after arrival and gradually declined towards the end of the production cycle. BRD already occurred immediately after arrival, but peaked 3 weeks after arrival. A long tail in the treatment curve was observed, associated with chronic pneumonia (relapse rate= 10.2% on average). It could be concluded that, whereas the group of digestive diseases was responsible for most mortalities, BRD was the single leading cause. BRD was also the main cause of morbidity, and a long tail of chronic disease, associated with BVDV at necropsy, was observed.

Next, the impact of BRD, diarrhoea, otitis and arthritis on mortality and carcass traits was studied in a sample of 3519 white veal calves (10 cohorts) (**Chapter 4.2.**). Disease registration was based on individual treatment by the producer and cause of death was determined by necropsy. Carcass data (hot carcass weight (HCW), color and fatness degree) were obtained from the slaughter houses. Average daily gain (ADG), carcass value (CV) and CV reduced by treatment costs were calculated. In comparison to non-treated calves, calves treated once for BRD showed a 0.066 kg/day reduction in ADG and 8.2 kg reduction of HCW, had a lower carcass value, a lower fatness degree and an increased mortality risk. The reduction in ADG, HCW, CV, fatness degree and the mortality risk increased dramatically with increasing number of BRD treatments. Calves treated multiple times for BRD also had higher odds for low carcass quality and were more likely to be classified as too red meat for white veal production. Arthritis increased the mortality risk, but reduced HCW only when associated with BRD. Otitis did not affect any of the studied parameters. Diarrhoea increased the mortality risk, reduced ADG and HCW by 0.078 kg/day and 8.8 kg, respectively, and decreased carcass quality. It could be concluded that even under intensive coverage by oral antimicrobial group treatments, substantial economic losses occur in calves, which require individual treatment for BRD, diarrhoea or arthritis. Controlling calf health by effective preventive and therapeutic strategies and in particular the prevention of chronic BRD is key for the profitability of veal operations.

Because of the importance of BRD in morbidity, mortality and the associated drug use, further focus was on the identification of the pathogens involved in the BRDC of white veal calves in Belgium.

In a fourth study (**Chapter 5.1.**), the prevalence of respiratory pathogens in diseased, unvaccinated, routinely medicated veal calves was determined, by sampling 24 natural outbreaks of BRD in Belgium (15 herds). Bacteria were cultured from nasopharyngeal swabs and seroconversion against viruses and *Mycoplasma bovis* was determined on paired sera. The inclusion criterion for sampling, was 10% of the animals with respiratory symptoms. Most outbreaks (75%) occurred between week 2 and 4 after arrival. In 54.2% of the outbreaks (13/24), antimicrobial group treatments for BRD had already been installed before the sampling criterion was reached, and in 29.2% (7/24) calves were still receiving oral antimicrobials at sampling. At the individual calf level, *Mycoplasma spp.*, *Mannheimia haemolytica* and *Pasteurella multocida* were retrieved from 70.5%, 21.5% and 26.0% of the swabs, respectively. At the herd level the presence of *M. bovis* could be confirmed on 84.6% of the examined herds. Serological evidence of BVDV, parainfluenzavirus type 3 (PI-3), bovine respiratory syncytial virus (BRSV), bovine adenovirus 3 (BAV-3), bovine coronavirus (BCV) and bovine herpesvirus 1 (BHV-1) infection was present on 71.4%, 53.3%, 40.0%, 46.7%, 30.0% and 26.7% of the herds, respectively. At necropsy, *Mycoplasma spp.* could be cultured from 61.9% of pneumonic lungs (n=21) and respectively 60.0% and 20.0% of the tested calves were BVDV (n=20) and BRSV (n=16) PCR positive. It can be concluded that BRD in white veal calves is of slow progressive nature rather than massive acute outbreaks, likely due to the presence of maternal immunity and the frequently applied metaphylactic antimicrobial therapy. Under such conditions the peak incidence is reached on average at week 3 post arrival. At that time, next to a variable viral component in the individual calf, (multi)resistant Pasteurellaceae are prevalent. Overall, *M. bovis* and BVDV appear to play an important role in both the initiation of BRD (acute outbreak) as in lethal chronic cases.

In a final study (**Chapter 5.2.**), the seroepidemiology of respiratory infections along the white veal production cycle and their association with BRD and carcass traits was determined. Also, the potential value of total and respiratory pathogen specific antibody levels at arrival to predict subsequent BRD and suboptimal carcass traits was evaluated. A total of 467 calves, housed in 15 veal herds, were sampled at arrival and 6, 12 and 24

weeks later. Antibody levels to 7 respiratory pathogens were determined at each time point by semi-quantitative ELISA. Circulation of PI-3, BAV-3 and *M. bovis* was detected in all studied herds. For BVDV, BRSV, BHV-1 and *M. haemolytica* 93.3% (13/15), 80.0% (12/15), 53.3% (8/15) and 86.7% (13/15) of the herds were infected, respectively. The respiratory disease peak in the first 6 weeks after arrival was associated with seroconversion to *M. bovis* (44.2% of the calves) and BVDV (32.0%). More than 50% of the calves seroconverted to at least one virus in this period, whereas seroconversion to *M. haemolytica* was not associated with the BRD peak. The prevalence of calves, persistently (PI) and transiently infected with BVDV, was 0.6% and 1.5%, respectively. PI calves had very low survival chances. With exception of *M. bovis* (10,7% of the calves on average), maternal antibodies were abundantly present at arrival for most pathogens, with little variation between herds. A worrisome 40.1% of the calves on average, ranging from 24.0 to 59.9%, had low total IgG levels at arrival, suggesting failure of passive transfer. Calves, seropositive to PI-3 and *M. bovis* at arrival, had a decreased and increased risk for BRD, respectively. Low (<8g/L) total IgG at arrival tended towards an increased BRD risk. No associations with carcass traits were found. Under the current medical management, the serostatus for respiratory pathogens at arrival was of little practical use to classify veal calves according to BRD risk or to predict carcass traits. To help improve the validity of epidemiological studies in the veal industry, standard case definitions should be provided and group antimicrobial treatments avoided.

In the general discussion (**Chapter 6**), first, the value of the applied study methodology for veal calf health monitoring systems and future holistic studies is discussed. With the obtained insights into the epidemiology of veal calf diseases (predominantly BRD) in mind, the general discussion mainly aimed at critically reflecting on the current (medical) management practices. A series of possible short and long term measures to control BRD and reduce antimicrobial use, to be evaluate in the future, is proposed.

Overall, it could be concluded that BRD is the main cause of morbidity and mortality in veal calves and subsequently the main indication for the highly intensive drug use in the Belgian veal industry. Despite the frequent antimicrobial coverage, the economic consequences of disease in calves requiring individual treatment were devastating. Almost every BRD pathogen was present on each herd, with a viral component in over

50% of the calves. Overall *M. bovis* and BVDV were most prevalent in acute and chronic BRD in white veal calves. The high prevalence of calves with insufficient levels of IgG at arrival is worrisome and requires attention at the herds of origin. Both the applied monitoring techniques as the obtained results can guide the veal industry in their search for evidence based preventive and therapeutic protocols, reducing antimicrobial use, while maintaining production results and animal welfare standards. More in specific, modifying current medical management towards *M. bovis*, exclusion of PI animals from veal production, vaccination and optimization of maternal immunity might be beneficial measures to reduce the BRD incidence and herewith associated antimicrobial use in veal calves.

SAMENVATTING

De vleeskalverindustrie is gespecialiseerd in het afmesten van hoofdzakelijk stierkalveren op een kunstmelkdieet met lage ijzergehaltes. Het resulterende wit kalfsvlees is een internationaal gesmaakt product van hoge kwaliteit. Net zoals bij andere voedselproducerende dieren werden recent hoge aantallen resistente bacteriën geïsoleerd uit vleeskalveren. Deze hoge antimicrobiële resistentieniveaus zijn uiterst zorgwekkend, doordat ze mogelijks therapiefalen bij mens en dier in de hand kunnen werken. Daarom riepen de Europese autoriteiten recent op tot het documenteren en herzien van het gebruik van antimicrobiële middelen bij voedselproducerende dieren. De druk op de diergeneeskunde om voorzichtiger en meer verantwoord met antimicrobiële middelen om te gaan is momenteel dan ook bijzonder groot.

Als algemene inleiding (**Hoofdstuk 1.1**) wordt een overzicht gegeven van de productiekenmerken en uitdagingen eigen aan de Belgische vleeskalversector. Een samenvatting van de huidige kennis over antimicrobiële resistentie bij pathogene, zoönotische en commensale bacteriën van vleeskalveren wordt gegeven. Wereldwijd is *bovine respiratory disease* (BRD of ademhalingsstoornissen) de grootste oorzaak van morbiditeit en mortaliteit bij jongvee in bijna elk rundveeproductiesysteem. BRD is een multifactoriële ziekte die ontstaat uit de complexe interactie van pathogenen met omgevings- en gastheerfactoren (immunititeit). Daarom wordt dit ziektecomplex ook *bovine respiratory disease complex* (BRDC) genoemd. Het BRDC verschilt van productiesysteem tot productiesysteem en kennis over de epidemiologie en pathogenese van BRD is nodig voor elk productiesysteem apart om effectieve controlematregelen voor dat productiesysteem te kunnen bepalen. In **hoofdstuk 1.2** wordt een overzicht gegeven van het BRDC bij vleeskalveren. Uit dit literatuuroverzicht bleek dat vleeskalveren een uitermate hoog risico op BRD hebben, waardoor antibioticumgebruik noodzakelijk is om ziekte te voorkomen. Er is bijna geen informatie beschikbaar over de pathogenen, die verantwoordelijk zijn voor BRD bij vleeskalveren.

Om economische redenen en door het ontbreken van epidemiologische informatie over de verschillende ziektes bij vleeskalveren, zijn dierenartsen al tientallen jaren lang aangewezen op empirische, vaak blinde, behandelingen in de vleeskalversector. Omwille van deze problematische situatie en door de opkomst van antibioticumresistentie bij vleeskalveren, was het overkoepelende doel van dit doctoraat inzicht te verwerven in het huidige antibioticumgebruik, in de epidemiologie van morbiditeit en mortaliteit en

in de onderliggende pathogenen van BRD bij Belgische witvleeskalveren (**Hoofdstuk 2**). Deze objectieve gegevens kunnen de vleeskalversector bijstaan in het bedenken en evalueren van duurzame preventieve en therapeutische protocollen in de toekomst, leidend tot een verminderd antibioticumgebruik.

Momenteel zijn er geen gegevens over het gebruik van antimicrobiële middelen bij witvleeskalveren in België beschikbaar. Daarom was het eerste doel van dit doctoraat het in kaart brengen van het antimicrobieel en anti-inflammatoir medicatiegebruik in de Belgische vleeskalverindustrie (**Hoofdstuk 3**). Er werd een prospectieve studie op 15 vleeskalverbedrijven uitgevoerd, met als doel het monitoren van groeps- en individuele behandelingen. Behandelingsincidenties (TI) gebaseerd op de standaard dierdagdosering (ADD), de voorgeschreven dierdagdosering (PDD) en de werkelijk gebruikte dierdagdosering (UDD) werden berekend. Het antibioticumgebruik was zeer hoog met gemiddelde 416.8 dierdagdoseringen per 1000 dieren *at risk*. Het totale antibioticumgebruik bestond voor 95.8% uit orale groepsbehandelingen en slechts voor 4,2% uit individuele behandelingen. BRD was de hoofdindicatie voor zowel groepsbehandelingen (53.0%) als individueel antibioticumgebruik. De meest frequent gebruikte antimicrobiële middelen in groepsbehandelingen waren: oxytetracycline (23.7%), amoxicilline (18.5%), tylosine (17.2%) en colistine (15.2%). Er werd frequent afgeweken van de dosissen, vermeld in de bijsluiters, met onderdosering in 47.3% van de groepsbehandelingen (vnl. oxytetracycline en tylosine om dysbacteriose te behandelen). Gebaseerd op de UDD, gebruikten kleinere integraties (<50 bedrijven) meer antibiotica dan grotere. Bedrijven met maar 1 veehouder gebruikten hogere doseringen in de groepsbehandelingen. Een heel grote variëteit aan antimicrobiële middelen werd gebruikt om kalveren individueel te behandelen, waaronder de *critically important* cephalosporines (8.0%) en fluoroquinolones (12.6%). Individuele behandelingen werden hoofdzakelijk overgedoseerd. Anti-inflammatoire middelen werden veel minder gebruikt ($TI_{UDD} = 5.94$), hoofdzakelijk groepsbehandelingen met natriumsalicylzuur. Anti-inflammatoire middelen die individueel geïnjecteerd werden, werden over het algemeen overgedoseerd. De belangrijkste conclusie was dat antimicrobiële middelen op dit moment zeer intensief gebruikt worden in de Belgische vleeskalversector. Er kan enkel verminderd worden als men het aantal groepsbehandelingen drastisch reduceert.

In een tweede studie (**Hoofdstuk 4.1.**) werden de oorzaken en de epidemiologie van morbiditeit en mortaliteit bepaald bij Belgische witvleeskalveren van het melktype, vleestype of kruisingen. In totaal werden 5853 kalveren op 15 bedrijven (5 per type) gedurende één productiecycclus gevolgd. De oorzaken van mortaliteit werden doormiddel van lijkschouwing bepaald. Morbiditeit werd dagelijks genoteerd door de veehouders. Het totale sterftepercentage was 5,3% en was significant hoger bij kalveren van het vleestype t.o.v. het melktype of kruisingen. De meest voorkomende oorzaken van mortaliteit waren pneumonie (1.3% van de kalveren), pensstoornissen (0.7%), idiopathische peritonitis (0.5%), enterotoxaemie (0.5%) en enteritis (0.4%). Idiopathische peritonitis is een ziekte van onbekende oorzaak, die specifiek voorkwam tussen 8 en 10 weken na aankomst. Kalveren van het Belgisch witblauwe ras hadden hogere kansen om te sterven door pneumonie, enterotoxaemie en arthritis. Het aantreffen van het bovine virale diarree virus (BVDV) op lijkschouwing was geassocieerd met chronische pneumonie en pleuritis. In totaal werd 25.4% van de kalveren individueel behandeld, resulterend in een totale incidentiedichtheid van morbiditeit van 1.7 gevallen per 1000 kalfdagen *at risk*. De incidentiedichtheid van BRD, diarree, arthritis en otitis was 0.95, 0.30, 0.11 en 0.07 gevallen per 1000 kalfdagen *at risk*, respectievelijk. Morbiditeit was het hoogst in de eerste drie weken na aankomst, en verminderde gradueel naar het einde van de productiecycclus toe. Er waren reeds gevallen van BRD onmiddellijk na aankomst, maar de hoogste incidentie werd waargenomen 3 weken na aankomst. De behandelingscurve toonde een lange uitloper die geassocieerd was met chronische pneumonie (10.2% hervallers). Er werd geconcludeerd dat waar gastro-intestinale ziekten verantwoordelijk waren voor de grootste mortaliteit, BRD de individuele hoofdoorzaak was. BRD was ook met voorsprong de hoofdoorzaak van morbiditeit, gekenmerkt door een grote chroniciteit die geassocieerd was met BVDV op lijkschouwing.

Vervolgens werd de impact van BRD, diarree, otitis en arthritis op mortaliteit en karkaskenmerken bestudeerd in een steekproef van 3519 witvleeskalveren (10 bedrijven) (**Hoofdstuk 4.2.**). Morbiditeitsregistratie was gebaseerd op individuele behandeling door de kalverhouder en de oorzaak van mortaliteit werd bepaald d.m.v. lijkschouwing. Karkasgegevens (warm karkasgewicht (HCW), kleur en vetheidsgraad) werden via de slachthuizen bekomen. Dagelijkse groei (ADG), karkaswaarde (CV) en CV verminderd met de behandelingskosten werden berekend. T.o.v. onbehandelde

kalveren, vertoonden kalveren, éénmalig behandeld voor BRD, een vermindering in groei en HCW van respectievelijk 0.066 kg/dag en 8.2 kg. Deze dieren hadden ook een lagere karkaswaarde, een lagere vetheidsgraad en een merkelijk hogere mortaliteit. Naarmate het aantal behandelingen voor BRD toenam, werden de vermindering in dagelijkse groei, karkasgewicht, karkaswaarde, vetheidsgraad en het risico op sterfte steeds groter. Dieren die meerdere keren voor BRD moesten behandeld worden hadden eveneens grotere kansen op een minderwaardige karkaskwaliteit met een te donkere vleeskleur om als wit kalfsvlees vermarkt te kunnen worden. Arthritis vergrootte het risico op sterfte, maar reduceerde het karkasgewicht alleen maar als BRD tegelijkertijd aanwezig was. Otitis beïnvloedde geen enkele van de bestudeerde parameters. Diarree verhoogde het risico op sterfte, verminderde de dagelijkse groei met 0.078 kg/dag en het karkasgewicht met 8.8 kg. Er kon geconcludeerd worden dat zelfs wanneer orale groepsbehandelingen met antibiotica intensief gebruikt werden, er grote economische verliezen optraden bij kalveren die nog individuele behandeling voor diarree, BRD en arthritis nodig hadden. Het controleren van de gezondheid van vleeskalveren d.m.v. efficiënte preventieve en therapeutische strategieën, en in het bijzonder de preventie van chronische BRD, is essentieel voor de winstgevendheid van vleeskalverbedrijven.

Omwille van het belang van BRD in morbiditeit, mortaliteit en het daarmee geassocieerde antibioticumgebruik, werd er verder gefocust op het identificeren van de pathogenen die betrokken zijn bij het BRDC van witvleeskalveren in België.

In een 4^{de} studie (**Hoofdstuk 5.1.**) werd de prevalentie van ademhalingspathogenen bij zieke, niet-gevaccineerde en regelmatig gemedicineerde vleeskalveren bepaald. Er werden 24 BRD uitbraken op 15 bedrijven bemonsterd. Nasopharyngeale swabs voor bacteriologie en gepaarde sera voor virussen en *Mycoplasma bovis* werden genomen. Het inclusiecriteria voor staalname was een minimum van 10% van de dieren met respiratoire symptomen. De meest uitbraken (75%) kwamen tussen week 2 en 4 na aankomst voor. In 54.2% van de uitbraken (13/24), waren er reeds groepsbehandelingen met antibiotica ingesteld voor het bereiken van het staalnamecriteria. Op 29.2% (7/24) van de uitbraken stonden de kalveren zelfs op antibiotica op het moment van staalname. Op het individuele kalfniveau, konden *Mycoplasma spp.*, *Mannheimia haemolytica* en *Pasteurella multocida* geïsoleerd worden uit respectievelijk 70.5%, 21.5% en 26.0% van de swabs. Op bedrijfsniveau kon de

aanwezigheid van *M. bovis* bevestigd worden op 84.6% van de onderzochte bedrijven. Serologisch bewijs van infectie met BVDV, parainfluenzavirus type 3 (PI-3), bovien respiratoir syncytieel virus (BRSV), bovien adenovirus 3 (BAV-3), bovien coronavirus (BCV) en bovien herpesvirus 1 (BHV-1) was aanwezig op respectievelijk 71.4%, 53.3%, 40.0%, 46.7%, 30.0% en 26.7% van de bedrijven. Op lijkschouwing konden *Mycoplasma spp.* uit 31.6% van de longen met pneumonie gekweekt worden en respectievelijk 60.0% en 20.0% van de geteste kalveren was BVDV of BRSV positief. Er kon besloten worden dat BRD bij vleeskalveren traag progressief is, eerder dan te bestaan uit massale acute uitbraken. Mogelijke verklaringen zijn de aanwezigheid van maternale immuniteit en het frequente gebruik van metafylactische antimicrobiële therapie. De incidentiepiek wordt onder zulke omstandigheden gemiddeld bereikt 3 weken na aankomst. Op dat moment zijn er, naast een variabele virale component in het individuele kalf, (multi)resistente Pasteurellaceae aanwezig. In het algemeen, lijken *M. bovis* en BVDV een belangrijke rol te spelen in zowel de initiatie van BRD (acute uitbraken) als in lethale, chronische gevallen.

In een laatste studie (**Hoofdstuk 5.2.**) werd de epidemiologie van respiratoire infecties bij witvleeskalveren gevolgd gedurende de productiecycclus. Associaties tussen seroconversies voor de 7 bestudeerde pathogenen en het risico op BRD en de karkassenmerken werden onderzocht. Daarnaast werd ook de potentiële waarde van het bepalen van de serostatus voor respiratoire pathogenen en het gehalte aan (maternale) antistoffen bij aankomst voor het voorspellen van BRD bekeken. In totaal werden 467 kalveren op 15 bedrijven bemonsterd bij aankomst en na 6, 12 en 24 weken. Antistoffengehaltes t.o.v. 7 respiratoire pathogenen werden bepaald met semi-kwantitatieve ELISA's. Circulatie van PI-3, BAV-3 en *M. bovis* kon aangetoond worden op elk bedrijf. BVDV, BRSV, BHV-1 en *M. haemolytica* werden op respectievelijk 93.3% (13/15), 80.0% (12/15), 53.3% (8/15) en 86.7% (13/15) van de bedrijven gedetecteerd. De hoogste incidentie van BRD werd waargenomen in de eerste 6 weken en was geassocieerd met seroconversie t.o.v. *M. bovis* (44.2% van de kalveren) en BVDV (32.0%). Meer dan 50% van de kalveren seroconverteerde t.o.v. ten minste 1 virus in die periode. Seroconversie t.o.v. *M. haemolytica* gebeurde hoofdzakelijk later dan 12 weken na aankomst en was dus niet geassocieerd met de BRD piek. De prevalentie van kalveren die permanent (PI) of transiënt geïnfecteerd zijn met BVDV bij aankomst was respectievelijk 0.6% en 1.5%. IPI's hadden zeer lage overlevingskansen. Met

uitzondering van *M. bovis* (gemiddeld was 10.7% van de kalveren positief bij aankomst), waren maternale antistoffen t.o.v. de bestudeerde respiratoire pathogenen overvloedig aanwezig bij aankomst, met weinig variatie tussen de bedrijven. Gemiddeld genomen had een zorgwekkende 40.1% van de kalveren totale antistofgehaltenes (IgG) die lager waren dan 10 g/L. Deze gehaltenes zijn onvoldoende hoog en sterk suggestief voor onvoldoende of slecht getimedede colostrumopname op het bedrijf van herkomst. Kalveren die bij aankomst seropositief waren voor PI-3 of *M. bovis*, hadden respectievelijk een verlaagd en verhoogd risico om individueel behandeld te worden voor BRD. Er was een trend voor een hoger BRD risico bij kalveren met totale IgG gehaltenes lager dan 8 g/L. Er konden geen associaties tussen de diverse pathogenen en de karkassenmerken aangetoond worden, als er gecorrigeerd werd voor het al dan niet voorkomen van BRD. Met het huidige niveau van antibioticumgebruik, bleek het bepalen van de serostatus t.o.v. respiratoire pathogenen van weinig praktische nut voor het voorspellen van BRD of karkassenmerken. Om de validiteit van epidemiologische studies in de vleeskalverindustrie te verbeteren, is er een grote noodzaak aan het invoeren van standaarddefinities voor de diverse ziektes en dient het verstrekken van groepsbehandelingen vermeden te worden.

In de algemene discussie (**Hoofdstuk 6**), wordt eerst de waarde van de gebruikte methodologie voor het ontwikkelen van gezondheidsmonitoringsprogramma's en het uitvoeren van holistische studies in de vleeskalversector besproken. Gebaseerd op de door dit doctoraat bekomen inzichten in de epidemiologie van de verschillende ziekten bij vleeskalveren (hoofdzakelijk BRD), wordt er in de algemene discussie kritisch gereflecteerd op het huidige (medicamenteuze) gezondheidsmanagement. Een reeks van mogelijke korte en lange termijn maatregelen om in de toekomst BRD beter te controleren en het gebruik van antimicrobiële middelen te verminderen wordt voorgesteld.

Als algemeen besluit kon gesteld worden dat BRD de hoofdoorzaak is van morbiditeit en mortaliteit bij witvleeskalveren en hierdoor ook de hoofdindicatie is voor het zeer intensieve gebruik van antimicrobiële middelen in de Belgische vleeskalverindustrie. Ondanks het veelvuldige gebruik van orale antimicrobiële middelen, zijn de economische gevolgen van ziekte bij dieren die individuele behandeling nodig hebben dramatisch. Bijna elk BRD pathogeen was aanwezig op elk bedrijf, met een virale

component in meer dan 50% van de kalveren. *M. bovis* en BVDV waren de meest voorkomende pathogenen, zowel bij acute als chronische BRD gevallen. De hoge prevalentie van kalveren met onvoldoende totale antistoffengehaltes bij aankomst is zeer zorgwekkend en vraagt grotere aandacht op de bedrijven van herkomst. Zowel de gebruikte monitoringstechnieken als de bekomen resultaten kunnen de vleeskalversector begeleiden in hun zoektocht naar *evidence based* preventieve en therapeutische protocollen die het gebruik van antimicrobiële middelen reduceren, maar tegelijk het huidige productie- en dierenwelzijnsniveau behouden. Meer specifiek, zouden het aanpassen van het huidige medicamenteuze management naar *M. bovis* toe, het niet meer opzetten van IPI's, vaccineren en het optimaliseren van de maternale immuniteit gunstige maatregelen moeten zijn om de BRD incidentie en het hiermee geassocieerde gebruik van antimicrobiële middelen te verminderen.

CURRICULUM VITAE

Bart Pardon werd geboren op 13 oktober 1983 te Tielt. Na het beëindigen van het secundair onderwijs aan 'De Bron' te Tielt, richting Latijn-Wetenschappen, startte hij in 2001 met de studies diergeneeskunde aan de Universiteit Gent. Hij behaalde in 2007 het diploma van dierenarts (optie herkauwers) met grootste onderscheiding. Zijn afstudeerwerk (Prokinetica bij runderen: het gebruik van erythromycine bij pensdrinkers- Promotor: Prof. Dr. P. Deprez) en studieresultaten werden respectievelijk bekroond met de prijs voor de beste scriptie m.b.t. herkauwers en de prijs van de faculteit diergeneeskunde.

Onmiddellijk na afstuderen trad hij in dienst van de vakgroep Interne Geneeskunde en Klinische Biologie van de Grote Huisdieren (UGent) als voltijds assistent, onder begeleiding van prof. Dr. P. Deprez en prof. Dr. G. van Loon. Hij legde zich toe op de inwendige ziekten en gezondheidszorg van herkauwers en stond, naast dienstverlening voor de kliniek, in voor het klinische onderwijs aan de laatstejaarsstudenten. Hij nam eveneens deel aan de nacht- en weekenddiensten van de vakgroep. Daarnaast was hij betrokken bij diverse Veepeiler projecten van Dierengezondheidszorg Vlaanderen, o.a. over Boviene Neonatale Pancytopenie en ademhalingsstoornissen bij vleeskalveren. Gesteund door deze onderzoeksresultaten begon hij in 2008 aan zijn doctoraatswerk over het belang van ademhalingsstoornissen in morbiditeit, mortaliteit en antibioticumgebruik bij vleeskalveren. In 2011 vervulde hij de doctoraatsopleiding.

Bart Pardon is auteur of medeauteur van meerdere publicaties in nationale en internationale wetenschappelijke tijdschriften, was meermaals spreker op (inter)nationale congressen en is reviewer voor verschillende veterinaire tijdschriften.

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