

Papers

Prevalence of respiratory pathogens in diseased, non-vaccinated, routinely medicated veal calves

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The prevalence of respiratory pathogens in diseased veal calves was determined in 24 respiratory disease outbreaks in 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* species, *Mannheimia haemolytica* and *Pasteurella multocida*, were isolated from 70.5 per cent, 21.5 per cent and 26.0 per cent of swabs, respectively. At the herd level, the presence of *M bovis* could be confirmed in 84.6 per cent of the herds examined. Seroconversion against bovine viral diarrhoea virus (BVDV) was present in 71.4 per cent of herds, parainfluenzavirus type 3 in 53.3 per cent, bovine respiratory syncytial virus in 40.0 per cent, bovine adenovirus type 3 in 46.7 per cent, bovine coronavirus in 30.0 per cent, and bovine herpesvirus type 1 in 26.7 per cent. At postmortem examination, *Mycoplasma* species could be cultured from 61.9 per cent of pneumonic lungs (n=21). Sixty per cent of calves tested were positive for BVDV (n=20), and 20.0 per cent were positive for bovine respiratory syncytial virus (n=16).

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 and others 2003). The microbial aetiology includes viruses and bacteria (including Mollicutes) and their relative contribution varies according to geographical region and production system. The aetiology is well documented in several production systems such as North American feedlots, dairy calf rearing and beef cow-calf operations (Allen and others 1991, 1992, Van Donkersgoed and others 1993, Ganaba and others 1995, Haines and others 2001, Fulton and others 2000, 2002, Thomas and others 2002, Schahriar and others 2002, Härtel and others 2004, Gagea and others 2006a, b, Hägglund and others 2006, Booker and others 2008, Angen and others 2009, Gulliksen and others 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 and others 1992, Rusvai and Fodor 1998, Autio and others 2007, Caswell and Archambault 2007, Arcangioli and others 2008, Radaelli and others 2008).

The white veal industry is specialised 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 characterised as the dominant pathogen (Arcangioli and others 2008) and in an Italian study, all veal calves were seropositive for *M bovis* at slaughter (Radaelli and others 2008). No information on the pathogens involved in BRD outbreaks in the most frequent production system in Europe (non-vaccinated veal calves, four to eight animals/pen on a slatted 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 and others 2005, 2007b, Di Labio and others 2007). The objective of the present field study was therefore to determine the prevalence of respiratory pathogens in non-vaccinated, routinely medicated veal calves suffering from clinical BRD.

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Materials and methods

Animals and housing

The study was carried out between September 2007 and January 2009 on farms housing white veal calves, with an all-in all-out management in Flanders (Northern Belgium). Calves had origins from different European countries (predominantly Belgium) at a mean (sd) age of 14.8 (4.5) days on arrival, after a passage through a sorting centre. The animals were housed in individual boxes on slatted floors during the first six weeks. After this period, the metal framework of these individual boxes was removed and the animals were group-housed (four to eight animals per pen). The diet consisted of low-iron milk replacer, concentrates and fibre-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 per cent of

TABLE 1: General information and antimicrobial group treatments for 24 bovine respiratory disease (BRD) 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) Dox (40-45)
2	2	BB	52	685	1(6)	20	Otc+Tyl (10)	Otc+Tyl (0-10)
3	3	HF	52	555	2(12)	26	Amox+Col (10)	Col+Flum (5-15)
4	4	BB	46	325	2(10)	24	Amox+Col (10)	Tyl (8-13)
5	5	HF	52	167	3(20)	34	Amox+Col (5)	Dox (13-18)
6	6	HFxBB	52	650	8(54)	68	Otc+Col (10)	Dox+Tyl (35-40) Amox (36-46) Dox+Tyl (35-40)
7	7	HF	52	580	6(40)	54	Otc+Col (10)	Dox+Tyl (35-40)
8	8	BB	46	250	7(46)	60	TS+Col (10)	Amox (16-26)
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)
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)	Amox (26-36)
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)

Amox Amoxicillin, BB Belgian Blue, Col Colistin, Dox Doxycycline, HF Holstein-Friesian, HFxBB Crossbreed, Otc Oxytetracycline, Til Tilmicosin, TS Trimethoprim-sulfonamide, Tyl Tylosin, Flum Flumequine

the calves with respiratory disease. At that time, 10 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 four of the following six clinical signs were present: fever ($>39.5^{\circ}\text{C}$), nasal discharge, spontaneous cough, increased respiratory rate (>45 breaths per minute), depression and anorexia. Calves treated individually within a week before the acute sampling time, were not sampled. Veterinarians were also asked to submit dead calves from the studied herds for postmortem examination. In total, 24 outbreaks on 15 (5.2 per cent) of the 287 veal farms in Flanders were included (Belgian cattle registration system data – Animal Health Service Flanders). In these herds, 240 diseased calves were sampled in total. Details on the 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 (Cattray and others 2005, 2008). Samples were transported at 4 to 7°C and processed within 24 hours at the laboratory (Animal Health Service Flanders). Isolation of bacteria (Pasteurellaceae and *Mycoplasma* species) was performed according to standard protocols described by Cattray and others (2007a, 2008). Briefly, for Pasteurellaceae, swabs were streaked on a Columbia blood agar (Oxoid) to which 5 per cent sheep blood and 16 $\mu\text{g}/\text{ml}$ bacitracin were added, incubated aerobically at 37°C (for *Histophilus somni* 7 per cent CO_2 was added), and further identified according to Quine and others (1994). *Mycoplasma* species were isolated on two modified pleuropneumonia-like organism agars (Difco). For each outbreak, a selected number of isolates (one per calf, up to three calves per herd) were stored at -18°C . Following this, strains were cultured again, and further identification of *M. bovis* was carried out using tDNA PCR on a selected number of isolates (Stakenberg and others 2005). Antimicrobial susceptibility testing was only performed for isolates from outbreaks that occurred while the calves were receiving oral antimicrobials. The Kirby Bauer disk diffusion technique, as described by Cattray and others (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, sulfonamide-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) 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 to BRSV (BRSV ELISA kit; Bio-X), parainfluenzavirus type 3 (PI-3) (Parainfluenza 3 ELISA kit), bovine adenovirus type 3 (BAV-3) (Adenovirus 3 ELISA kit), bovine coronavirus (BCV) (Coronavirus ELISA kit) and BVDV (SERELISA BVD p80 mono blocking; Synbiotics Europe). Additionally, serum from 155 calves (all herds except herds 8, 9, 10 and 11) was analysed by ELISA for antibodies to *M. bovis* (*Mycoplasma bovis* ELISA kit; Bio-X). Seroconversion was defined as an increase in signal of at least two magnitudes (0 to ++ or + to +++), according to the manufacturers guidelines. Antibodies to the gE antigen of bovine herpesvirus type 1 (BHV-1) were detected with an indirect ELISA (HerdChek* Anti-IBR-gE; IDEXX Laboratories). 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.

Postmortem examination

The postmortem examination was performed according to an in-house standard 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 and others 2004) and BRSV (Boxus and others 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) according to an in-house standard protocol. Briefly, 1 cm^3 of lung tissue was suspended in sterile phosphate buffered saline and centrifuged (20 minutes, 1500 g). The suspension was incubated for one hour

TABLE 2: Prevalence of respiratory pathogens in veal calves with bovine respiratory disease (BRD), at calf, outbreak and herd level

Sample	Agent	Calf level (n=219)		Outbreak level (n=24)		Herd level (n=15)	
		Percentage of positive samples (number positive/number sampled)	Percentage of positive outbreaks (number positive/number sampled)	Mean number of positive calves (range)	Percentage of positive herds (number positive/number sampled)		
Nasopharyngeal swab	<i>M haemolytica</i> [*]	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)		
	<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</i> species [†]	70.8 (155/220)	95.4 (21/22)	7 (0-10)	92.3 (12/13)		
	<i>M bovis</i> [†]	–	90.9 (20/22)	–	84.6 (11/13)		
Serum [‡]	<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)		
	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)		

* *Mannheimia haemolytica* sensu lato

† No species identification at the calf level. Identification (*Mycoplasma bovis* PCR) only for selected isolates per outbreak

‡ Seroconversion rates: seven 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 analysed, respectively

BAV-3 Bovine adenovirus type 3, BCV Bovine corona virus, BHV-1 Bovine herpesvirus type 1, BRSV Bovine respiratory syncytial virus, BVDV Bovine viral diarrhoea virus, PI-3 Parainfluenza virus type 3

(5 per cent CO₂, 37°C) with monolayers of bovine kidney cells. Cells were kept in culture for 12 days and then frozen (–20°C). After fixation of the cells with 4 per cent 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 to 2008 or winter 2008 to 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 herd effect (SPPS statistics v17.0; SPSS). Significance was set at $P < 0.05$.

Results

Clinical observations

The BRD outbreaks on the veal farms were typically of a slow progressive nature rather than sudden outbreaks. The first cases usually occurred within one week following arrival on the farm, but the sampling criterion (10 per cent of the animals with clinical BRD) was on average reached at 22.2 (15.0) days after arrival. Of the outbreaks, 4 per cent, 33 per cent, 29 per cent, 13 per cent and 21 per cent occurred at 1, 2, 3, 4 or more than 5 weeks after arrival, respectively. In 13 outbreaks, (11 of 15 herds [54 per cent]) oral group antimicrobial treatments for BRD had already been initiated before the sampling time (first BRD group treatment on average 14.2 [8.2] days after arrival). In seven (29 per cent) outbreaks (seven of 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

Polybacterial results (n=14) and *Proteus* species overgrowth (n=7) were considered as non-interpretable and therefore treated as missing values, leaving 219 swabs for analysis. Due to overgrowth *Mycoplasma* species cultures of herds 2 and 7 were also considered as missing values, leaving 220 swabs. From 87 per cent (190 of 219) of the swabs, a well-defined culture could be obtained and 75 per cent (179 of 219) yielded at least one respiratory pathogen. Commensal or contaminating flora was detected in 15 per cent of the swabs and involved *Staphylococcus aureus* (five of 219 [2.3 per cent]), *Escherichia coli* (19 of 219 [8.7 per cent]), *Pseudomonas aeruginosa* (two of 219 [0.9 per cent]), *Streptococcus* species (six of 219 [2.7 per cent]) and *Enterobacter* species (one of 219 [0.5 per cent]). 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 per cent (six of 219) car-

ried both *P multocida* and *M haemolytica* in the nose, 22.6 per cent (46 of 203) carried *P multocida* and *Mycoplasma* species, 16.2 per cent (33 of 203) carried *M haemolytica* and *Mycoplasma* species, and 2.5 per cent (five of 203) carried all three pathogens. The prevalence of *Mycoplasma* species was significantly higher in animals sampled at younger ages in comparison with animals sampled at older ages (more than five weeks after arrival) ($P < 0.05$). No other significant risk factors could be identified on the present dataset. In three of the seven 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 one to seven calves per outbreak, and in two outbreaks (herds 7 and 14), only *P multocida* (one and two 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 for *P multocida* and *M haemolytica*) (herd 14 not tested).

Serology

Convalescent sera were not available for seven calves for reasons of death, leaving 233 paired sera. In 40.3 per cent (94 of 233) of the calves, seroconversion to 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, 10 and 3, respectively. At the outbreak level, on average seroconversion against two viruses (range zero to four) was detected. No viral component was identified only in outbreak 6. At the individual calf level, multiple viral infections were rare (13 of 233 [5.6 per cent]) and predominantly involved BVDV (nine of 13) and BAV-3 (nine of 13). Only three (1.3 per cent) calves seroconverted for three or more viruses. Among the 155 calves for which *M bovis* serology was available, 32.9 per cent seroconverted to *M bovis*. Of these 155 calves, 21.3 per cent also seroconverted to at least one virus (BVDV [5.2 per cent], PI-3 [3.9 per cent], BCV [3.9 per cent], BAV-3 [2.6 per cent], BHV-1 [1.9 per cent] and BRSV [1.9 per cent]). The prevalence of seronegatives in acute serum and the changes in serological status are given in Table 3. Of the calves, 4.3 per cent, 24.0 per cent, 27.9 per cent, 24.9 per cent, 14.2 per cent, 4.3 per cent and 0.4 per cent were seropositive in the acute serum to all six, five, four, three, two, one or no viruses, respectively.

Postmortem examination

A total of 21 calves from eight different herds underwent postmortem examination. The average age was 62.9 (24.0) days, which was on average 29.1 (26.0) days after the acute sampling date. Details on the postmortem examinations are given in Table 4. *Mycoplasma* species (61.9 per cent), *P multocida* (9.5 per cent), *M haemolytica* (9.5 per cent)

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

Agent*	Percentage of seronegative calves (number seronegative/number sampled)		Percentage of calves seroconverting (number seroconverted/ number sampled)	Percentage of calves seronegative in acute serum that seroconverted (number seroconverted/ number seronegative)
	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)

* BAV-3 Bovine adenovirus type 3, BHV-1 Bovine herpesvirus type 1, BCV Bovine coronavirus, BVDV Bovine viral diarrhoea virus, BRSV Bovine respiratory syncytial virus, PI-3 Parainfluenzavirus type 3

and *Arcanobacterium pyogenes* (28.6 per cent) could be isolated from the lungs. Of the examined calves (n=20), 60.0 per cent were BVDV PCR positive on pulmonary or spleen tissue, whereas 20.0 per cent of the examined calves (n=15) were BRSV PCR positive.

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 and others 2007). Most respiratory bacteria are ubiquitous and can be detected in both healthy and ill calves by nasopharyngeal swab and bronchoalveolar lavage (BAL) (Allen and others 1991, Autio and others 2007). At the group level, a good association between cultures from nasopharyngeal swabs and BALs has been demonstrated for *M haemolytica*, *P multocida* and *M bovis* (Allen and others 1991, De Rosa and others 2000, Godinho and others 2007).

In the diseased veal calves, the nasal prevalence of *P multocida* was lower (26.0 per cent v 33.6 per cent) and of *M haemolytica* higher (21.5 per cent v 5.9 per cent) than in a previous study on healthy veal calves in Belgium (Catry and others 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 and others 1992, Haines and others 2001, Shahriar and others 2002, Arcangioli and others 2008). The fact that, in seven herds, calves were undergoing 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 five of these seven 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 and others 2001, Shahriar and others 2002, Gagea and others 2006b, Arcangioli and others 2008, Radaelli and others 2009). On the contrary, in Norwegian, Finnish and Danish dairy calves, *M bovis* was not found (Autio and others 2007, Angen and others 2009, Gulliksen and others 2009). In 84.6 per cent 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 per cent) (Arcangioli and others 2008). Of the examined calves, 32.9 per cent seroconverted to *M bovis* during acute BRD. In previous studies, between 60 and 100 per cent of the calves seroconverted between arrival and one or two months later, respectively, and at slaughter all calves were seropositive (Arcangioli and others 2008, Radaelli and others 2009). In the present study, 32.3 per cent of the calves were already seropositive in the acute sample (three weeks after arrival), compared with only 2.2 per cent at arrival

in the study of Arcangioli and others (2008). This difference can be explained either by a higher level of maternal antibodies to *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 postmortem examination, the typical caseonecrotic pneumonia, associated with *M bovis* infection, was seen in several cases. *Mycoplasma* species were isolated from 61.9 per cent of the cases examined postmortem, and *M bovis* was identified in all calves in which species identification was performed (n=10). In North American feedlots, *M bovis* was isolated from 82 to 92 per cent of pneumonic lungs at postmortem examination (Haines

and others 2001, Shahriar and others 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 postmortem examination.

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 and others 2002). The commercial antibody ELISAs 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 acute serum, 81 per cent 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 and others 2001, Shahriar and others 2002, Gagea and others 2006a). At postmortem examination in the present study, the calves were also found to be predominantly chronic cases in which BVDV and *M bovis* could be frequently demonstrated. The number of acutely ill calves in which a simultaneous seroconversion for *M bovis* and BVDV could be demonstrated, was however, limited (eight of 155 [5.2 per cent]). BVDV was also frequently involved in multiple viral infections as described in feedlots and dairy calves (Richer and others 1988, Fulton and others 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 and others 2009). Nevertheless, the occurrence of seroconversion against BHV-1 on 26.7 per cent 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 two months later was determined, instead of related to the clinical period as in the present study (Arcangioli and others 2008). For BRSV, it is well known that maternal antibodies do not protect against infection and can suppress serum responses (Kimman and others 1987, 1988, Uttenthal and others 2000). Seroconversion of seronegatives in the acute serum is, however, useful and shows that only a minority (13.2 per cent) of seronegative calves seroconvert. Nevertheless, the virus was detected in 20 per cent of the examined calves at postmortem examination, whereas BHV-1, BAV-3 and PI-3 were not. The presence of BRSV was confirmed on 40 per cent 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 per cent vs 13 per cent (Autio and others 2007) and 19 per cent (Nikunen and others 2007)). Of the seronegative calves in the acute serum, 40.7 per cent seroconverted against BCV. Although seroconversion does not distinguish between a respiratory

TABLE 4: Postmortem examination findings, bacteriology and virology in 21 veal calves with fatal bovine respiratory disease (BRD)

Calf ID	Outbreak ID	Days after arrival	Age (days)	Postmortem examination findings	Bacteriology (culture)	Virology			BAV (isolation)	PI-3 (isolation)
						BRSV (PCR)	BVDV (PCR)*	BHV-1 (PCR)		
1	1	43	57	Chronic catarrhal bronchopneumonia	<i>Mycoplasma</i> species <i>Mycoplasma haemolytica</i> <i>Escherichia coli</i>	-	+	-	-	-
2	1	52	65	Chronic catarrhal bronchopneumonia	<i>E coli</i> <i>Streptococcus</i> species	-	+	-	-	-
3	3	12	26	Subacute catarrhal bronchopneumonia	<i>Arcanobacterium pyogenes</i>	+	+	-	-	-
4	3	50	64	Chronic catarrhal bronchopneumonia Hydranencephalia	<i>Pseudomonas aeruginosa</i>	-	+	-	-	-
5	3	50	64	Chronic caseonecrotic pneumonia Pleuritis	<i>Mycoplasma</i> species <i>A pyogenes</i>	-	+	-	-	-
6	3	50	64	Chronic catarrhal bronchopneumonia	<i>Mycoplasma</i> species <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>Mycoplasma bovis</i> <i>M haemolytica</i>	-	+	-	-	-
9	9	69	83	Chronic catarrhal bronchopneumonia	<i>P aeruginosa</i> <i>E coli</i> <i>Proteus</i> species	+	-	-	-	-
10	9	88	102	Chronic caseonecrotic pneumonia Pleuritis	<i>M bovis</i>	-	+	-	-	-
11	9	92	106	Chronic caseonecrotic 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</i> species	+	-	-	-	-
13	15	20	34	Chronic caseonecrotic pneumonia Pleuritis	<i>Proteus</i> species	-	+	-	-	-
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</i> species	ND	+	ND	ND	ND
19	23	65	79	Chronic bronchopneumonia + abscess	<i>M bovis</i> <i>Proteus</i> species	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 caseonecrotic pneumonia	<i>M bovis</i> <i>A pyogenes</i>	ND	+	ND	ND	ND

* BVDV PCR on lung tissue for all calves, except calves 17 to 21 (spleen)

- Negative, + Positive, BAV Bovine adenovirus, BHV-1 Bovine herpesvirus type 1, BRSV Bovine respiratory syncytial virus, BVDV Bovine viral diarrhoea virus, ND Not determined, PI-3 Parainfluenzavirus type 3

or intestinal infection, involvement of BCV in respiratory disease is likely as concurrent faecal and nasal shedding occurred in 38 per cent of infected calves in a previous study (Hasoksuz and others 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. Under such conditions, the BRD peak incidence is on average reached at week three after 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 per cent 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 economical result and the reduction of antimicrobial use in the white veal industry remains to be determined.

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Prevalence of respiratory pathogens in diseased, non-vaccinated, routinely medicated veal calves

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